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IN

HEALTH AND TYPE - 2 DIABETES MELLITUS

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BONAFIDE CERTIFICATE

This is to certify that this dissertation on

"A STUDY ON LIPOPROTEIN(a)

IN

HEALTH AND TYPE - 2 DIABETES MELLITUS"

submitted by **Dr.K.S.PREMKUMAR** is a work done by him during the period of study in this department from August 2003 to September 2006.

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INTRODUCTION

Lipoprotein (a) has been identified as a major risk factor of atherosclerosis in non - diabetic and diabetic patients. It is a well known fact that diabetic patients have a high risk of cardiovascular disease; Lp(a) has been recognized as the high risk factor of the above disease in diabetic patients.

Lp(a) is an LDL-like particle to which apolipoprotein(a) [apo(a)] is attached through a disulfide bond to apolipoprotein B100 [apoB100]. Genetic variation of the Lp(a) genes is the main determinant of Lp(a) serum levels but non genetic factors could also affect its concentration. Several studies have shown the influence of diet, drugs and hormones on Lp(a) levels. There are several studies that evaluated the relationship between Lp(a) and glycaemic control in diabetic patients but little is known about the influence of lipid profiles on serum Lp(a) concentration. Some authors have found a positive correlation between serum levels of Lp(a) and LDL cholesterol in non - diabetic patients. There is also a negative correlation between Lp(a) and triglycerides that has been reported in nondiabetic subjects, but there are no specific studies on this issue in the diabetic population.

Hence with a keen interest to determine the relationship between Lp(a) and other lipid parameters in Normal and Type 2 Diabetic subjects in our part of the country this work has been taken up for the study.

REVIEW OF LITERATURE

Lipoprotein (a) [Lp(a)] was detected in 1963 by Kareberg in Norway in a study designed to detect antigenic variation in Human LDL. He could distinguish between so called Lpa +ve and Lpa -ve serums and demonstrated Autosomal Dominant inheritance of Lp(a) trait using antibodies raised in rabbits against human lipoprotein preparation. Shortly it became apparent that Lp(a) by itself is a distinct particle rather than an allelic variant of LDL[17,18,19,20].

As Lp(a) was rediscovered several times, several names like "SINKING PRE β LIPOPROTEIN"[21], "PRE β LIPOPROTEIN"[22], "LDL a-1" or just "A NEW ATYPICAL LIPOPROTEIN" have been attributed to it[17,19,23].

A breakthrough in the research of the lipoprotein was when Mclean et al., in 1987 cloned and sequenced one of its components apo(a) which revealed a high degree of homology to plasminogen[17,19,24,25]. Lipoprotein(a) has been identified as a major risk factor of atherosclerosis in non diabetic and diabetic patients[1,2] due to this component apo(a).

STRUCTURE OF Lp(a)

Lipoprotein(a) [Lp(a)] is a low density lipoprotein (LDL) like particle formed by the association of the highly polymorphic glycosylated apolipoprotein(a) [apo(a)] through a disulphide bond with apolipoprotein B100 (apoB100)[3], the classic protein moiety of LDL[26]. Lp(a) is found to be spherical with the size of 4 million daltons and a diameter of 250 A°[3]. Lipoprotein(a) has a

density from 1.040 to 1.30 g/ml[19]. In Agarose gel electrophoresis, Lp(a) moves as a single band between LDL and VLDL[19,40]. Genetic variation of the Lp(a) genes is the main determinant of Lp(a) serum levels but non - genetic factors like diet, drugs and hormones[5,6,7,8] could also affect its concentration. The schematic structure of lipoprotein(a) is given in Figure No.1.

Although apo(a) transcripts have recently been found in adrenal glands, lungs, pituitary, brain and testes[31], it is mainly synthesized by the liver as a precursor with lower molecular mass which is processed into the mature form and then secreted into the blood stream[32]. After secretion, free apo(a) binds rapidly to circulating LDL's to generate complete Lp(a) particles[33]. The assembly of Lp(a) as per the study of Rath M Niedorf et al.,[62] is produced in the endoplasmic reticulum of liver though in another study by Lobentanz EM et al Lp(a) was hypothesized to be assembled almost exclusively extracellularly, as no apo(a) - apo B100 complexes were detected by them within cells[32].

The composition and physiochemical properties of the lipoprotein remnant known as Lpa -ve (Chart No.1) derived after dissociation of apo(a) from Lp(a) by chemical reduction has made clear that Lp(a) can be considered as a genetically determined variant of LDL increased in density and size. Lp(a) belongs to the heterogenous family of cholesterol enriched lipoprotein. Cholesterol in either free or esterified form represents almost 40% of its mass. The relative weight of phospholipids (17-24%) is comparable to that of proteins (17-29%) whereas the triglyceride content is rather limited, usually below 9%[34,39]. Almost 23% of the apo(a) mass is attributable to N and O glycosides producing the remarkable

electronegative potential of the lipoprotein particle [Lp(a)][37].

About 90% of Lp(a) concentration is under genetic regulation. The greatest part of the variability in Lp(a) levels (over 40%) is accounted for by quantitative polymorphism in the internal sequence of the apo(a) gene. Qualitative polymorphism in the sequence of the promoter play only a minor role (from 10 to 14%)[41]. Despite this genetic regulation, some metabolic abnormalities may have effect on Lp(a) levels in plasma[42,43]. Among these acute phase response, hormonal homeostasis, diabetes, liver and renal failure and defects in the LDL receptor gene have all been shown to influence the still enigmatic metabolism of this lipoprotein.

Lp(a) has been attributed a polymorphic structure, the reasons for which are enumerated below :

1. The composition of Lp(a) and its lipid contents
2. The structure of apo(a)
    a. Size polymorphism of apo(a)
    b. Sequence polymorphism of apo(a)

1. COMPOSITION OF Lp(a) AND ITS LIPID CONTENTS

Lp(a) is a complex assembled from two different components which forms the central and outer core[17,19,40]. The central core which is hydrophobic is formed by LDL. The lipid fraction of LDL is constituted by esterified cholesterol, phospholipid, triglycerides, free cholesterol and apoB100, its protein fraction. The structure of Lipoprotein(a) is shown in Figure No.2.

The outer core of Lp(a) is found to be apo(a) which is hydrophilic glycoprotein with a uniquely high degree of conserved internal repeat structure and an enormous size heterogenicity[19,40]. Hence while LDL contains only apoB100, Lp(a) contains both apo B100 and apo(a). In other words Lp(a) differs from LDL mainly by addition of the large glycoprotein apo(a) which is bound to apoB 100 by disulphide linkage[19].

In some subject Lp(a) contains 2 molecules of apo(a) per molecule of apo B 100. In such cases, the density of Lp(a) depends not only on size of apo(a) but also on its number[19,44].

G.Lippi and G. Guidi have made evident in their Table (Chart No.1) where they have compared Lp(a) and LDL that the two molecules are not identical because Lp(a) is much large with a greater protein content and higher density than LDL.

2. STRUCTURE OF Apo(a)

This is a glycoprotein with varying degrees of glycosylation (approximately 35% by weight). The carbohydrate fraction is constituted by sialic acid and "N" and "O" linked carbohydrate chains where N-Acetyl glucosamine is the principal "N" linked sugar. Its molecular mass is about 230 - 280 kDa (kilodaltons). Apo(a) is composed of a kringle containing domain and a serine protease domain[45,46,47,48,49] as shown in Figure No.3.

The basic modular units of apo(a) are the structures called kringles which are sequences of 80 - 90 aminoacids. They are non catalytic cysteine rich

internally looped structures with the shape of a "Pretzel". As the shape resembles a danish cake called kringle the basic units of apo(a) have been named so. These kringles (k) are internally stabilized by 3 cross linking disulphide bridges[17,19] as illustrated in Figure No.4.

The kringle structures has been discovered not only in apo(a) but also in certain other protein molecules namely plasminogen, tissue plasminogen activator (t-PA), urokinase, prothrombin, protein C, coagulation factors VII, IX, X, XIII[17,19]. Though the kringles in the above enumerated proteins are of 5 types referred to by Roman numerals I-V, apo(a) contains only 2 types of kringles namely type IV and V[17,18,19]. Each kringle structure of apo(a) contains a potential "N" linked glycosylation site formed by Asp-Leu-Thr[17].

As the number of times a specific kringle is repeated identically in a protein molecule it is represented by Arabic Numerals. The repetition of 36-40 times of type IV kringle in apo(a) is represented as KIV(n) where (n) represents the number of units of KIV. The kringles are serially numbered from 1-37 from the NH_2 terminal end of apo(a). It is to be noted that out of the 37 kringles, the kringle with serial number 1-36 are of KIV which is followed by a single kringle of Type V serial No.37[19,20].

Among the kringles in KIV, 10 basic types designated KIV type 1-10 are seen in apo(a) which are similar to each other but not identical to each other. All the types present in apo(a) are all in a single copy except KIV Type 2 which is present in a variable number of copies ranging from 3-40. The varying number of KIV Type 2 repeat is said to be a major determinant of apo(a) size

heterogenicity giving origin to a large number of apo(a) isoforms[20,25,49].

The KIV repeats of apo(a) is followed by a single KV. Thus the whole kringle domain of KIV repeat and KV of apo(a) contains together 11 different type of kringles out of which only KIV type 10 contains all the key interactive aminoacids to bind to lysine of fibrin and thereby interferes with the similar function of plasmin involved in fibrinolysis. Interindividual differences in the aminoacid sequences of apo(a) KIV Type 10 are also known to exist[19]. It is also said that the penultimate KIV present at the carboxyterminal of apo(a) has an unpaired cysteine residue that favours the disulphide bond formation with the cysteine residue in apoB100[17].

As reviewed already kringle structures is common to several proteins which are considered to be members of a protein superfamily and are found to be regulatory proteins in both fibrinolytic and coagulation systems. Among the proteins plasminogen is said to have striking homology to apo(a) due to similarities of several features between the two compounds. Inspite of the homology, apo(a) does not possess the proteolytic activity of the active form of plasminogen namely plasmin which results in fibrinolysis since the preactivation peptide and the kringles I-III present in the latter are absent in apo(a)[50]. Similarly while the active site of plasminogen present in the protease domain is formed by Arg-Val that in apo(a) has Ser-Val.

The difference with the active site of the two components is said to result in the abscence of any cleavage of apo(a) to a form which will have proteolytic activity similar to plasmin. However in 1992 an alternative site for proteolytic

action for apo(a) has been suggested by Guerva et al.,[17] thereby suggesting a protease activity to apo(a) different from that of plasmin.

ISOFORMS OF Apo(a)

In 1987, 6 isoforms of apo(a) of different masses ranging from 400-800kDa were demonstrated by Uttermann and coworkers[19,51]. When compared to apoB100 the relative mobilities of apo(a) categorized into 3 types of isoforms based on electrophoretic mobility in comparison to apoB100 which are faster than apoB100, similar to apoB100 and 4 types slower than apoB100 which are S_1, S_2, S_3, S_4 were recognized. The (Chart No.2) also gives the number of KIV repeats, molecular weight, size and the concentration of the isoforms in the blood.

GENETIC BACKGROUND OF Apo(a) AND Lp(a)

While Lp(a) is said to be inherited as an Autosomal Dominant trait, apo(a) is considered to be a superfamily of Trypsin like serine protease which consists of 4-proteases namely apo(a), plasminogen and hepatocyte growth factor I and II[18].

The ability to synthesize apo(a) is confined to a restricted group of primates; however, the insectivore hedgehog produces an apo(a) like protein composed of multiple tandem repeats of a plasminogen kringle III homologous domain but lacking the protease domain[52].

The human apo(a) gene is located in a gene cluster within 400kb of genomic DNA on the telomeric region of chromosome 6(6q 26-27)[53,54] including the sequence encoding apo(a), plasminogen and other two pseudogenes with

highly homologous untranslated 5^{l} flanking regions[55]. Three additional homologous genes designated as plasminogen - related genes have been identified unlinked to the apo(a) gene cluster and resident on chromosome 2 and 4.

The apo(a) gene belongs to a puzzling gene family that includes several similar sequences encoding prothrombin, tissue - type plasminogen activator (t-PA), urokinase A - chain, plasminogen, coagulation factor XII, Macrophage stimulating factor, hepatocyte growth factor and other unclear function[56]. Nucleotide analysis of human genes encoding these proteins reveals that sequences of exon and relative boundaries differ only from 1 to 5% and that the types of exon / intron functions and the positions of introns in the sequences are almost identical. These data suggest that the genes might have developed during recent primate evolution from a common ancestral component of the kringle - related serine proteases, most likely plasminogen via duplication and exon shuffling[52].

The apo(a) gene shares the highest homologies with the gene of the zymogen plasminogen. The sequence encoding for plasminogen kringle V domain is retained, whereas the plasminogen kringle IV domain encoding sequence, exists in multiple variable tandem repeats. In contrast, apo(a) lacks the sequences of plasminogen preactive region and plasminogen kringle domains I through III despite the strong genetic homologies, a single point mutation in the sequence of the protease domain deprives apo(a) of most of plasminogens enzymatic properties.

Apo(a) and plasminogen genes are about 50 kb apart and are oriented in

opposite directions. The leader sequence of apo(a) is separated from the first kringle of apo(a) by an ~14kB intron. In the variable apo(a) KIV repeat domain the coding region for one KIV is split into 2 exons of 162bp (EXON-1) and 180bp (EXON-2) by the 4.2 kb intron 1. Between the 3^{l} end of 2nd exon and 5^{l} end of next exon is a 1.4 kb intron 2. There are multiple copies of Exon 1 and Exon 2 encoding KIV repeats. The total size of genomic KIV repeats is 5.6 kb. The high degree of internal KIV repeats in "Complementary DNA" of apo(a) suggests that the size polymorphism of the protein may be due to inherited differences in the number of KIV repeats in the gene. The high quantitative polymorphism in the sequence encoding the plasminogen KIV Type 2 domain explains the high degree of individual allelic size polymorphism of the protein, as to date no fewer than 34 size allele have been identified in the apo(a) locus, encoding as many detectable isoforms in plasma[52,59]. The autosomal codominant inheritance of apo(a) isoforms are said to be controlled by a series of a autosomal alleles Lp^{F}, Lp^{B}, Lp^{S1}, Lp^{S2}, Lp^{S3}, Lp^{S4} at a single locus. A seventh allele Lp^{0} (null allele) has been found to control the null type isoform where there is no detectable isoforms[19].

The size of apo(a) particle usually determines its rate of hepatic synthesis and secretion; Null alleles, producing virtually no detectable circulating Lp(a), can be frequently observed. The molecular basis of these null allele seems to be an inframe 47 amino acid delection in the sequence of the protease domain that hinders the correct splicing of mRNA and generates a defective protein, irregularly subjected to a sequence of intracellular rearrangements which are essential for processing and secretion of complete and functional particles[60].

Among these, the trimming of N-linked glucoses which occurs after the folding of the protein into the endoplasmic reticulum, is thought to be a critical process[61].

SYNTHESIS OF Apo(a) AND Lp(a)

Various studies have shown that apo(a) is formed as a precursor with a molecular weight more than the mature secreted protein. Most intracellular apo(a) as per the study of Gerd Utermann exists as a precursor of ~400kDa in the endoplasmic reticulum and is free unassembled with apoB100 : but the mature intracellular form of apo(a) exists as 700kDa form in Golgi fraction and is assembled with apoB100[19]. As per the study of Rath M Niedorf A et al.,[62]. Lp(a) is produced in the endoplasmic reticulum of liver where it is presumably linked to apoB100. In the endoplasmic reticulum and later in the Golgi apparatus apoB100 is loaded with triglyceride for secretion as very low density lipoprotein. Although the density of most Lp(a) falls between the densities of low and high density lipoprotein, Lp(a) can circulate with triglyceride rich lipoproteins and is presumably secreted as such[63,64].

Apo(a) mRNA is said to be present in liver, testes, brain but is most abundant in liver[17,19]. Among the organs, liver alone produces apoB100. Hence the potential to assemble apo(a) into Lp(a) is said to be present only in liver[65].

Though the above data give the idea that Lp(a) is formed within the liver cell alone, extracellular assembly is said to be certainly possible. Various studies done with transgenic mice and humans have shown that Lp(a) is also formed in plasma by the association of LDL with apo(a) secreted into plasma in the free

form. Apo(a) in Lp(a) is believed to be derived from a pool that is metabolically different from the pool from which LDL is found.

FUNCTIONS OF Lp(a)

Various functions have been attributed to Lp(a) which are enumerated below :

I. Tissue Repair

II. Inhibition of fibrinolysis

III. Effect on Atherogenesis

IV. Inhibition of cancer growth and spread

V. Acts as a surrogate for ascorbate

I. Tissue Repair

It now seems likely that Lp(a) offered an evolutionary advantage to humans by promoting and accelerating the healing of wounds and the repair of tissue injuries and vascular lesions.

Lp(a) behaves as an acute phase reactant. The sequence of the apo(a) gene contains interleukin - 6 (IL-6) responsive elements that enhance transcription of the gene[150]. IL-6 generates a marked, dose dependent enhancement of apo(a) mRNA sysnthesis that leads to the accumulation of Lp(a) particles in hepatocyte culture[151(.

Due to the additional presence of apo(a), Lp(a) can be recognized by a broad variety of receptors at the surface of endothelial cells, macrophages, fibroblasts and plateletes[152,153]. Defensin, a peptide, released from activated or

senescent neutrophils, enhance the binding of Lp(a) to endothelial cells by approximately four fold and to smooth muscle cells by six fold[154]. Although it is not yet clear whether Lp(a) particles are internalised directly or instead by prior, extracellular degradation, the large amount of cholesterol carried by the lipoprotein can easily be extracted and used at the site of its accumulation.

Lp(a) binds to several compartments of the vascular wall and the subendothelial matrix[155]; this binding is partially mediated by the lysine binding sites (LBS) of its apo(a) moiety[153]. High affinity bindings to fibronectin, fibrinogen, glycosaminoglycans and proteoglycans were observed in the presence of Ca^{2+} and Mg^{2+} ions; further weaker interactions were described with laminin and beta - 2 glycoprotein I, but no binding was observed to von wille brand factor, vitronectin or collagen Type IV[155,156,157].

Accumulation of Lp(a) molecules has been demonstrated in the arterial walls of human coronary and cerebral vessels[158], aorta and peripheral arteries. In those sites, the relative amount of apo(a) deposition is significantly related to the extent of atherosclerosis. Large amounts of Lp(a) can be demonstrated in growing atherosclerotic plaque and vein grafts[159]. In growing atherosclerotic lesions the accumulation of apo(a) in degraded, free and intact (but oxidized) forms appear to be preferential to that of other apolipoprotein[160]. This process might be attributed to the tendency of apo(a), to bind to connective tissue elements such as proteoglycans, glycosaminoglycans and especially fibronectin[155].

The cellular uptake and degradation of Lp(a) follows several pathways as Lp(a) particles bind to a wide variety of cellular receptors[161,162,163,164,165,166] and

other unrecognized endosomal membrane proteins[163]. This binding process is promoted by lipoprotein lipase or sphingomyelinase[167]. Lipoprotein lipase enhances the cell association of Lp(a) five fold and the consequent cellular degradation by about three fold[168] whereas the oxidative modification of Lp(a) results in avid uptake by monocyte - macrophages[169]. The affinity of Lp(a) to triglyceride rich lipoproteins and LDL's and the strong molecular interactions with several components of the endothelial matrix might further enhance the catabolism of Lp(a) by alternative as yet unclear pathways, promoting accelerated internalisation and degradation of cholesterol rich lipoproteins[170]. Lp(a) particles are susceptible to oxidative modification and scavenger receptor uptake, leading to intracellular cholesterol accumulation and foam cell formation[171,172] which contributes further to atherogenesis. The raised sialic content of Lp(a) is thought to contribute to the oxidative resistance of the native particle[172]. Finally increased Lp(a) levels are associated with a selective impairment of vasodilator capacity of receptor mediated endothelial stimuli, contributing to the pathogenesis of myocardial ischaemia[173].

II. Inhibition of Fibrinolysis

Lp(a) displays unequivocal growth factor like properties promoting growth of human umbilical vein endothelial cells (hUVECs) in synergy with basic fibroblast growth factor and insulin[174] and enhancing the proliferation of human vascular smooth muscle cells (hVSMCs) in culture by inhibiting the activation of transforming growth factor. These observation are not surprising in view of the fact that apo(a) belongs to a family of growth factors evolved from a common

ancestral kringle containing serine protease, including the hepatocyte growth factor / scatter factor (HGF/SF) a potent effector in promoting growth, movement and differentiation of epithelial, endothelial and the hepatocytic growth factor / macrophage stimulating protein (HGF1/MSP) an effector of macrophage chemotaxis and phagocytosis[175]. Role of Lp(a) in Tissue Repair is summarized in (Chart No.3).

As a vascular injury occurs, the acute phase response, concomitantly induced by the cellular release of several mediators including IL-6 stimulates the hepatic synthesis of newly formed apo(a) particles in the blood steam. Shortly afterwards apo(a) accumulates at the site of vascular injury as it binds to cellular receptors present in the surface of residual vascular cells, macrophages and platelets and also to the exposed subendothelial matrix and to immobilized fibrin. The large amount of apo(a) bound to the fibrin surface and endothelial cells, inhibits the lysis of clot.

The role of Lp(a) in inhibition of fibrinolysis is illustrated in Chart No.5. Though the extensive homology of apo(a) with plasminogen has raised the possibility that apo(a) may function similar to the former protein in the fibrinolytic process, it has been found that it is not so. Infact the reality is that apo(a) present in Lp(a) interferes with many steps in the complex biochemical cascade of reactions involved in fibrinolysis as illustrated in Chart No.5; the reactions leading to fibrinolysis are depicted in Chart No.4.

1. **As illustrated in the charts Lp(a) inhibits fibrinolysis by competing with plasminogen in the following manner[19,178,179,180]:**

a. Competition for plasminogen activation by tissue plasminogen activator (t-PA).

t-PA synthesized and released from endothelial cells, binds at a separate domain of Annexin II (multi domain amphipathic phospholipids binding protein and a unique endothelial membrane site for fibrinolytic assembly systems). The above multimolecular complex leads to a cell bound plasmin that contributes to the nonthrombogenic character of the endothelial cell surface. By interfering with this assembly via direct competition with plasminogen binding, Lp(a) downregulates endothelial cell plasmin generation and shifts the vessel surface to a more thrombogenic phenotype[17,18]. In addition Lp(a) also acts as a competitive inhibitor of t-PA in the presence of fibrinogen[17,19].

b. Competition for plasminogen binding to fibrinogen and fibrin.

Lp(a) may bind to fibrin via kringles in apo(a), thus delivering cholesterol to sites of recent injury and wound healing[17,181]. This binding of plasminogen to fibrin is normally mediated by lysine (fibrin) binding site of plasminogen KI. This binding is enhanced by plasminogen activators. Plasmin formed by activation induces modification in fibrin that in turn creates more plasminogen binding. Lp(a) competes with this binding site and inhibits the binding of plasminogen to fibrinogen and fibrin thus inhibiting fibrinolysis and promoting thrombosis.

c. Competition for plasminogen binding to cellular binding sites.

The fibrinolytic system in contact with the surface of endothelial cells play a critical role in thromboregulation. Glucose - plasminogen is the main circulating

fibrinolytic zymogen which binds specifically to plasminogen receptors at the endothelial cell surface thereby triggering an increase of several folds in plasmin generation by t-PA (by conversion of Glucose - Plasminogen to Lysine - Plasminogen). Lp(a) inhibits Glucose - Plasminogen binding to the endothelial cell receptors[17,19].

d. Competition for Plasminogen (PMN) binding to tetranectin

Tetranectin is a plasma protein which binds reversibly to KIV of plasminogen (PMN) and enhances plasminogen activation by t-PA. Lp(a) binds to tetranectin with high affinity whereby less plasmin is formed resulting in decreased fibrinolysis[17,19].

e. Enhancement of plasminogen activator inhibitor (PAI-1) activity

Lp(a) regulates expression of PAI-1. It increases the amount of PA1-1 activity[17,18,19]. All the above functions of Lp(a) are said to contribute to the proatherogenic property of Lp(a).

2. Lp(a) has also been found to aid in the formation of fibrin network where the proteins fibrin, fibronectin, fibrinogen and apo(a) are held as a mesh by cross linking between Endo γ glutamyl and Endo-ε-lysyl residues of the above protein[19]. The crosslinking of the above (protein) surface structures aid in the deposition of Lp(a) in the growing atherosclerotic plaques.

Hence from the inhibitory functions of Lp(a) on plasminogen it is clear that Lp(a) interferes with fibrinolysis but aid clot formation whereby the delicate balance between the complex cascade of reactions between clot formation and degradation is tilted towards the former.

III. Atherogenic effects of Lp(a)

The various modes by which Lp(a) contributes towards atherogenesis is given below and also illustrated and in Chart No.6.

a. Lp(a) has the capacity to bind to glycosaminoglycans; it is all the more trapped in atherosclerotic plaques, thus contributing to atherogenesis.

b. Lp(a) forms complexes with proteoglycans and are taken up by macrophages. Lp(a) which is converted to oxidized Lp(a) by polymorphonuclear leucocytes are also taken up by macrophages via scavenger receptors. Both lead to foam cell formation and cytokine production which acts as a chemoattractant and mitogen for smooth muscle cells.

c. Lp(a) interacts with platelets interfering with platelet aggregation.

d. Lp(a) by downregulating the generation of plasmin which normally activates transforming growth factors β (TGF-β) and thereby blocks smooth muscle proliferation is able to impair activation of TGF-β thereby contribute to smooth muscle cell proliferation.

e. Lp(a) is also said to decrease production of endothelial derived growth factor (EDGF) and increase production of adhesive glycoprotein intercellular adhesion molecule - I (ICAM-1).

Hence it is clear that apo(a) aid atherosclerosis not only by interfering with fibrinolysis and promoting a mesh of it with fibrin but also by its other functions which affect smooth muscles and EDGF. This is illustrated in Chart No.6.

In a nutshell it is said that the atherogenic effect of Lp(a) is due to the cholesterol delivery to the site of injury or to the endothelial cells, blocking of plasmin generation, endothelial cell modulation, smooth muscle cell proliferation and angiogenesis[18]. Lp(a) is said to cause neovascularization atherosclerotic plaque thus contributing to angiogenesis[18]. Marlys L Kochinsky, Ph.D., has identified the association of Lp(a) with atherothrombotic disease and has also further classified the potential mechanism by which Lp(a) increase leads to the atherosclerosis as proatherogenic and prothrombotic[192]. The same has been illustrated in Chart No.7.

IV. Lp(a) inhibits cancer growth and spread

Angiostat, a38kDa fragment generated by cancer mediated proteolysis of a plasminogen including plasminogen kringle domains I through IV inhibits neovascularisation of tumors and metastasis[184, 185]. Furthermore, recombinant form of plasminogen i.e. kringle V domain sharing high sequence homology with four kringles of angiostatin inhibits endothelial cell migration[186].

As most plasminogen derived kringles have strong inhibitory effect on angiogenesis[185,186] and as the polar kringle domains are highly homologous to plasminogen residues (77-100%)[187] it is quite conceivable that Lp(a) kringle fragments produced after physiological degradation of whole particles in vivo[188] have similar properties in anatoganizing or reducing growth and spread of cancer. The concentration of Lp(a) is commonly reported to be significantly increased in cancer patients as compared to health controls, irrespective of source and degree of the malignancy tumor[189]. However the clinical relationship between Lp(a) and cancer is still obscure.

V. Lp(a) is a surrogate for Ascorbate

According to the classic "Unified theory" of former Nobel prize winner Linus C Pauling and Mathias Rath, Human occlusive cardiovascular disease is a degenerative condition induced by chronic Ascorbate deficiency in which the large extracellular deposition of Lp(a) represents a powerful biological defence mechanism[190,191]. Thereby Lp(a) is regarded as surrogate for Ascorbate. No other reliable or biological evidence regarding the above topic has been published after the studies of Rath and Pauling.

SERUM LEVELS OF Lp(a)

Lp(a) is fully expressed in the first year life[70,71]. Plasma concentration is said to be heritable through kringle isoform transmission and is constant relatively throughout life[72]. It is said that due to the fact that both male and female sex hormones suppress Lp(a) there is lack of sex difference noted for the lipoprotein.

However Jacques Genest Jr et al.,[91] has given different reference range for males and females. Plasma Lp(a) concentration is said to vary from undetectable levels to 1 gm/dl[86]. There is strong evidence that Lp(a) levels are more dependent on synthesis of apo(a) than its catabolic rate. The distribution of Lp(a) in the population was highly skewed by Harlampos J Milionis et al., among population[123]. Approximately 90% of the population in England had serum levels less than 30mg/dl and occasionally patients had above 20,000 mg/dl[68]. As per Srinivasan SR et al.,[69] blacks had several fold higher Lp(a) than Asian and Caucasians. The pathological effects due to increased Lp(a) was noticed when it exceeded 30 mg/dl[87,88,72,89,69,90].

Reference plasma concentration of Lp(a) as determined by several workers in the field are given below.

1. James A Hearn et al.,[92] : Less than 4mg/dl
2. Jacques Genest Jr et al.,[91] : Male 13 - 14 mg/dl
   Female 14- 16 mg/dl
3. Berg et al., : 15-20 mg/dl
4. Harlampos J Milionis[123] : Less than 30 mg/dl
5. Bernard Cantin et al., : 32-34 mg/dl
6. Devanapalli[193] : 32.5 mg/dl for Asian Indians

Sex hormones and related compounds estrogen[73,74], progestin[75,76], estrogen - progestin combination[77], testosterone[78], anabolic steroids[78,76], tamoxifen[80], raloxifene[81] and corticotrophin and dexamethasone[82] have been found to lower Lp(a). Growth hormones have been shown to increase Lp(a).

Exercise, environmental factors, age and body mass index have been found to have little effect on Lp(a)[40,47,83,84,85]. But PB Duell et al.,[194] have found Lp(a) can be modulated by a complex interplay between insulin action, obesity, androgen levels and strenuous exercise[194].

Diets low in saturated fat and cholesterol had no effect on Lp(a) concentration[127] and studies of increased or decreased intakes of cholesterol and saturated and polyunsaturated n-6 and n-3 fatty acids similarly showed no or marginal effects on Lp(a)[128,129].

Certian dietary fatty acids may however affect Lp(a) concentration. Transmonounsaturated fatty acids constituting 5 to 10% of total energy intake increased Lp(a) by 20 - 70%[130,131] whereas transmonounsaturated fatty acids contributing 4% of energy intake and insignificant effects[132].

Fish-oil lowers Lp(a) concentration by 15% in normolipemic individuals Lp(a) concentration less than 200mg/dl[133] and by 37% hypertriglyceridemia patients[134] as it is said to decrease secretion of VLDL.

Plasma Lp(a) concentration were 25 - 33% lower when the subjects consumed a diet containing either palmitic acid or myristic acid + lauric acid then when they consumed a diet containing stearic acid[135].

Of the dietary proteins, plant proteins (particularly soy protein) have been found to lower atherogenic lipoproteins and sometimes increase antiatherogenic HDL as well.

Heavy alcohol consumption is said to lower Lp(a) and withdrawal causes rapid increase in Lp(a).

Of the hypolipemic drugs only neomycin sulphate[19,46,136,51,137,21] and nicotinic acid[138,139] decreased Lp(a) concentration substantially. Statins and bile acid sequestrants either had no effect or increased Lp(a)[144,145]. Fibrates appeared to lower Lp(a) to a modest degree[146,147].

CLINICAL SIGNIFICANCE

Lp(a) is an acute phase reactant[93,94]. Hence it is found to be increased in patients with cardiopulmonary bypass[17,48], on patients developing restenosis after PTCA, cerebrovascular disease[95,96,97,98], coronary atherosclerosis in cardiac transplant recipients[99], saphenous vein graft stenosis following bypass and peripheral arterial disease (PAD).

Lp(a) values can be increased as a part of cancer[100], menopause, hypothyroidism[101]. Lp(a) values can be decreased in liver failure[102] and hyperthyroidism[103]. Lp(a) when correlated with renal disease it is found to be increased in patients with Nephrotic Syndrome[104,105], (ESRD) end stage renal disease[106,107], in patients following renal transplantation in ESRD[108,109,110,111,112]. Increased serum Lp(a) is seen in both Type 1 and Type 2 Diabetic Patients[23,113,114,115].

CATABOLISM OF Lp(a)

This is said to take place mainly by 3 events[46]. They are :-

1. Oxidative events 2. Proteolytic events 3. Lipolytic events.

In addition to the above 3 events reductive processes have also been found to play a part in the catabolism of Lp(a). All the 4 processes of catabolism of Lp(a) have been illustrated in Chart No.8.

1. Oxidative Events

Catabolism of Lp(a) is found to be similar to that of LDL. Lp(a) is oxidized by polymorphonuclear leucocytes and oxidized Lp(a) is taken up by the scavenger receptor of macrophages. The binding of Lp(a) to the above receptors and also to cell surface receptors of smooth muscle cells, lymphocytes and endothelial cells seem to play a role in the regulation of intracellular cholesterol metabolism and also in the removal of Lp(a) from plasma. Though Lp(a) and LDL are assumed to bind to some type of receptors, there are said to be differences in affinity and binding capacity of the larger molecular weight of Lp(a)[45]. Suggestion of two different conformations of receptor - protein, one for Lp(a) and one for LDL are also available[45]. More details of the same however are yet to be known.

Though the above details have given an ideal that Lp(a) under normal conditions is bound to receptors and degraded by receptor system, the exact percentage of Lp(a) pool cleared by receptor pathway is yet to be known.

PROTEOLYTIC EVENTS

Pancreatic and leucocytic elastases and cleaves apo(a) to generate 2 fragments F_1 and F_2 which represents the N-terminal and C-terminal domain of apo(a) respectively. As F_2 remains bound to apoB100 of LDL by the disulphide linkage it is said to form a mini Lp(a) particle which is also depicted in Figure No.6.

F_1 Fragment has been reported in plasma and urine in normal conditions and has been found to be increased in some pathological conditions.

LIPOLYTIC EVENTS

Enzymes like secretory phospholipase A_2 and sphingomyelinase which are active in the arterial wall are said to act on Lp(a).

They are said to modify Lp(a) as well as LDL due to which the 2 molecules bind more readily to proteoglycans, lysine etc. The above effect of the enzyme on Lp(a) is said to be due to the exposure of a 2nd lysine binding site on apo(a).

DIABETES MELLITUS

DEFINITION

Diabetes mellitus is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. When fully expressed diabetes is characterized by fasting hyperglycaemia, but the disease can also be recognized during less overt stages, most usually by the presence of glucose intolerance, most usually by the presence of glucose intolerance. The effect of diabetes mellitus

include long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, heart and blood vessels. Diabetes may present with characteristic symptom such as thirst, polyuria, blurring of vision, weight loss and polyphagia and in its most severe forms, with ketoacidosis or non ketotic hyperosmolarity, which in the absence of effective treatment, leads to stupor, coma and death. Often symptoms are not severe or may even be absent.

The WHO criteria for the diagnosis of Diabetes is given below :

1. Classic symptoms and casual plasma glucose >200mg/dl.
2. Fasting plasma glucose $\geq$ 126mg/dl
3. 2 hour post load plasma glucose $\geq$ 200mg/dl during OGTT.

WHO has suggested that in a symptomatic patient a random plasma glucose value of 11.1 mmol/l (200mg/dl) or more is diagnostic.

This is also adequate for the asymptomatic patient if found on more than one occasion (and not due to an obvious hyperglycaemic stimulus such as glucose infusion in a surgical patient). If random glucose estimates show lower degrees of hyperglycaemia estimation of fasting glucose levels or an oral glucose test may be used.

Differences between plasma and whole blood glucose concentration and between capillary and venous levels are too often ignored. Whole blood values are about 10 to 15 percent lower than those of plasma and capillary values are 7 percent higher than venous values in the fasting state and 8 percent higher after a glucose load. (It should also be noted that many patients who fall within the lower

part of the diabetic range (as defined the WHO) are also only Chemical Diabetics in the sense that they have no symptoms and no evidence of diabetic tissue damage on examination).

ETIOLOGIC TYPES

The etiologic classification of diabetes mellitus currently recommended by WHO and ADA is presented in (Chart No.9). This classification differs considerably from the previously recommended classification, which used the terms insulin dependent diabetes and non-insulin dependent diabetes[195]. These terms, however, were frequently misused and at best classified patients based on treatment needs rather than on etiologic characteristics. The most common forms of diabetes mellitus named as Type 1 and Type 2 have been reviewed below.

TYPE 1 : DIABETES MELLITUS

Type 1 diabetes is the form of the disease due primarily to β - cell destruction. This usually leads to a type of diabetes in which insulin is required for survival. Individuals with type 1 diabetes are metabolically normal before the disease is clinically manifest, but the process of β-cell destruction can be detected earlier by the presence of certain autoantibodies. Type 1 diabetes usually is characterised by the presence of anti-GAD, anti-islet cell or anti insulin antibodies, which reflects the autoimmune process that have led to β-cell destruction. Individuals who have one of more of these antibodies can be subclassified as having type 1A, immune - mediated type 1 diabetes[196,197].

Particularly in nonwhites, type 1 diabetes can occur in the absence of

autoimmune antibodies and without evidence of any autoimmune disorder. In this form of type 1 diabetes, the natural history also is one of progressive disease with marked hyperglycaemia resulting in an insulin requirement for prevention of ketosis and survival. Such individuals are classified as having type 1B or idiopathic diabetes[198].

Type 1A diabetes show strong associations with specific haplotypes or alleles at the DQA - A and DQ - B loci of the human leukocyte antigen (HLA) complex[199]. The rate of β-cell destruction is quite variable, being rapid in some individuals, especially in infants and children, slower in adults. Some have modest fasting hyperglycaemia that can change rapidly to severe hyperglycaemia or ketoacidosis, and others, particularly adults, may retain some residual β-cell function for many years and have sometimes been termed as having "latent autoimmune diabetes"[200,201].

Individuals with type 1 diabetes have low undetectable levels of insulin and plasma C-peptide. Patients with type 1A diabetes are also more likely to have other concomitant autoimmune disorders, such as Graves disease, Hashimoto thyroiditis, Addison disease, vitiligo or pernicious anaemia.

TYPE 2 : DIABETES MELLITUS

Type 2 diabetes is the most common form of diabetes. It is characterized by disorders of insulin action and insulin secretion, either of which may be the prominent features.

Although the specific etiology of this form of diabetes is not known,

autoimmune destruction of the β - cell does not occur. Patients with Type 2 diabetes usually - have insulin resistance and relative, rather than absolute deficiency.

This form of diabetes is associated with progressive β-cell failure with increasing duration of diabetes[202]. Ketoacidosis seldom occurs spontaneously but can arise with stress associated with another illness such as infection.

Most patients with type 2 diabetes are obese when they develop diabetes, and obesity aggravates the insulin resistance. Type 2 diabetes frequently goes undiagnosed for many years because the hyperglycaemia develops gradually and in the earlier stages is not severe enough to produce the classic symptoms of diabetes; however, such patients are at increased risk of developing macrovascular and microvascular complications. Their circulating insulin levels may be normal or elevated yet insufficient to control blood glucose levels within the normal range because of their insulin resistance. Thus, they have relative, rather than absolute, insulinopaenia.

Type 2 diabetes is seen frequently in women who have a previous history of gestational diabetes and in individuals with other characteristics of the insulin resistance syndrome, such as hypertension or dyslipidemia. The risk of developing type 2 diabetes increases with age, obesity and physical inactivity. Type 2 diabetes shows strong familial aggregation, so that persons with a parent or sibling with the disease are at increased risk, as are individuals with obesity, hypertension or dyslipidemia and women with a history of gestational diabetes. The frequency of type 2 diabetes varies considerably among different racial or

ethnic subgroups. Persons of Native American, Polynesian or Micronesian, Asian - Indian, Hispanic or African - American descent are at higher risk than persons of European origin[203]. Although the disease is most commonly seen in adults, the age of onset tends to be earlier in persons of non - European origin. The disease can occur at any age and is now seen in children and adolescents[204,205].

METABOLIC DERANGEMENTS IN DM[206]

The derangements in carbohydrate, lipid and protein metabolism in DM and the consequent effects of those derangements are illustrated in Chart No.10.

Complications of DM

They are classified into Acute and Chronic Complication.

Acute Complications

1. Diabetic ketoacidosis (DKA)
2. Hyperosmolar nonketotic coma
3. Lactic acidosis

Chronic Complications

1. Microangiopathy of Retina and kidneys leading to retinopathy, Nephropathy respectively.
2. Neuropathy
3. Macroangiopathy - where there is hyperlipidemia, oxidized LDL and Lp(a) all leading to premature atherosclerosis and premature ischaemia heart disease.

4. Non enzymatic glycation of proteins.

INVESTIGATORY PARAMETERS[207]

I. BIOCHEMICAL PARAMETERS

1. Urine Glucose

Normally glucose does not appear in urine until plasma glucose is >10 mmol / L (180 mg/dl).

2. Blood Glucose

Fasting and postprandial blood glucose are more reliable. Postprandial blood sugar is useful to diagnose, monitor and screen the disease. When fasting blood sugar is greater than 126 mg% or postprandial blood sugar is greater than 200 mg% and above, disease is well confirmed. But when Fasting blood sugar (FBS) is between 110 - 126 mg% or Postprandial blood sugar (PPBS) is between 126 - 199 mg% the person should be subjected to OGTT.

3. Glucose Tolerance Test

* Oral glucose tolerance test
* IV glucose tolerance test

NORMAL OGTT

* Fasting blood glucose 80 - 110 mg%

In OGTT

a. Peak is within one hour and the level reached is less than 160 mg/dl

b. Level reduces and reaches fasting level by 2 hours.

c. None of the urine samples contain sugar or ketone bodies.

4. Urine Ketone Bodies (K.B.)

* Ace test and ketostix test are used.

5. Blood Ketones

(Acetone, Acetoacetic acid and β hydroxy butyrate). Normally increased only when Diabetes mellitus is severe with ketoacidosis. Acutest and ketostix are the methods of choice.

Normal Reference Range = < 0.2 mmol / L

6. Long Term indices of Glycaemic Control

a. Glycated Proteins

Increased concentrations of glucose in extracellular fluid (ECF) lead to nonenzymatic glycation of lysine residues of proteins. This is irreversible and until the protein is degraded is this present in glycated form. This concentration reflects the mean blood glucose level during the life of that protein.

i. Glycated Haemoglobin (HbA1c)

It is the condensation of glucose with the N-terminal valine residue of each β chain of HbA to form an unstable schiff base. It forms 80% of HbA1.

* Reflects the concentration of blood glucose over a period of past 60 days.
* Normal Reference Range (RR) → 4-6%. More than the RR signifies absence of glycaemic control during the past 60 days.

ii. Fructosamine

* Formed by interaction of glucose with ε-amino group on lysine residues of albumin.
* Reflects control over 3 weeks prior to its determination.
* Normal reference range → 205 to 285 mmol/L.

7. Microalbuminuria

* Signal of nearly reversible renal damage.
* 24 hours urine albumin estimation is done.
* Reference Range - 30 - 300 mg / 24 hours.

8. Blood Acid Base Status

* pH - altered in ketoacidosis. In diabetic ketoacidosis pH is acidic and along with pH' Na, K and osmolality is measured.

9. Blood Lactate Levels

* It is increased in Lactic acidosis
* Normal reference range → 5 - 12 mg/dl

10. Lipid Profile

* Total cholesterol increased
* TGL is raised (due to increased VLDL)
* VLDL is increased
* Lp(a) is increased (secondary to nephropathy, impaired TGL metabolism, glycosylation impairs its catabolism).
* TGL rich lipoprotein remnants increased

* Oxidized LDL and glycated LDL increased.

II. SERUM INSULIN AND C-PEPTIDE ESSAYS

III. IMMUNOLOGICAL PARAMETERS

i. Antibodies - islet cell antibodies
   * Insulin Auto antibodies
   * GAD antibodies
   * IA-2 antibody (protein tyrosine phosphatase) - denotes that the cause is autoimmunity.

ii. Genetic Markers

TREATMENT

The treatment which is to be instituted in Diabetes Mellitus from mild to severe forms include

i. Exercise

ii. Diet

iii. Drug therapy
   a. Sulphonyl ureas
   b. Biguanide
   c. Thiozolidinediones
   d. Alpha glycosidase inhibitors
   e. Meglitinide

iv. Insulin

Lp(a) IN DIABETES MELLITUS

There are several studies that evaluated the relationship between Lp(a) and glycaemic control in diabetic patients[9,10].

Several people belonging to the medical faculty who have worked with Lp(a) have determined that it increases in both types of Diabetes Mellitus. The increase was found with Diabetes Mellitus with or without microalbuminuria[116] where increased Lp(a) was found to be an independent risk factor for atherosclerosis.

Different views regarding the influence of glycaemic control on Lp(a) have been reviewed which are given below.

1. Wester Louis et al.,[208] determined that there was no statistical differences between Lp(a) levels of both types of Diabetes mellitus and healthy controls. They had proposed that Lp(a) concentration in Type 1 and Type 2 Diabetes Mellitus were independent of short term and long term glycometabolic control or the occurrence of microalbuminiuria, neuropathies or retinopathies.

2. CJ Chang et al.,[124] found that Lp(a) levels are not elevated in diabetic patients even in poorly controlled metabolic conditions.

3. A Perez et al.,[209] have found that in Type 1 diabetes mellitus patients, improvement of glycaemic control does not improve plasma Lp(a) concentration regardless of baseline Lp(a) levels and

the degree of glycaemic control.

4. SM Haffner et al.,[122] have arrived at the conclusion that Lp(a) levels changed with glycaemic control in Type 1 Diabetes mellitus patients.

5. JJ Couper et al.,[117] have obtained a rise in Lp(a) levels during puberty in Type 1 Diabetes Mellitus.

6. Durlach et al., have found that there was no significant difference in Lp(a) concentration in Type 2 Diabetes mellitus patients and control subjects.

7. O'Brien T et al.,[211] have determined that Lp(a) levels were significantly higher in Type 2 Diabetes patients than control subjects and no association was found between Lp(a) levels and glycaemic control or CAD.

8. N Waseef et al.,[119] have found significant elevation in both android obese and non - obese Type 2 diabetic patients regardless of glycaemic control.

9. FR Heller et al.,[120] have determined high levels of Lp(a) in insulin requiring Type 2 Diabetes mellitus patients and has reasoned out chronic hyperinsulinemia as the eventual causal factor.

10. WD Scheer et al.,[121] have determined that optimizing body weight and tight glycaemic control may beneficially influence Lp(a) values

in patients with Type 2 Diabetes mellitus. They also concluded that Lp(a) levels was higher in Type 2 diabetes mellitus patients who were treated with insulin when compared to those on sulphonyl urea therapy.

11. T Kikuchi et al.,[212] have said that improvement of glycaemic control by insulin therapy does not influence Lp(a) levels in Type 2 Diabetes mellitus patients independent of baseline values and the degree of glycaemic control reached; they have further stated that Lp(a) levels are independent of lipid levels in other lipoporteins after improved glycaemic control in Type 2 diabetes mellitus[213].

LIPOPROTEIN'S IN DIABETES MELLITUS

TYPE 1 DIABETES MELLITUS

Very Low Density Lipoproteins

Extreme elevations in VLDL have been recognized as being a common occurrence in diabetic ketoacidosis, the stage at which insulin concentrations are minimal[214]. On the other hand, VLDL levels may not be elevated in individuals with type 1 diabetes who are receiving adequate therapy. It is now well established that elevations in VLDL triglycerides in type 1 diabetes are often correlated with the degree of glycaemic control[215,216].

In people with untreated type 1 diabetes, the fractional catabolic rate for endogenous triglyceride is decreased[217], as is the clearance rate for exogenous

triglyceride[218]. Thus, when insulin deficiency is extreme, clearance is impaired because the activity of LPL is dependent on insulin. In the early stages of insulin deficiency, production of VLDL is increased, probably because of the increase in mobilization of free fatty acids. This enhanced hepatic secretion of VLDL falls off in the later stages of ketoacidosis because of the decrease in hepatic protein synthesis secondary to the insulin deficiency. During severe ketoacidosis when there is marked insulin deficiency, hypertriglyceridemia is caused primarily by a deficiency in LPL acitivty, and overproduction of triglycerides may not occur despite elevated levels of free fatty acids.

LOW DENSITY LIPOPROTEINS

LDL concentration appears to vary directly with the extent of hyperglycemia. LDL levels are increased in poorly controlled type 1 diabetes diabetes. However, in many individuals with type 1 diabetes, LDL concentrations are not different from those of age and weight matched controls.

In uncontrolled Type 1 diabetes, LDL fractional clearance is probably decreased because insulin appears to potentiate LDL binding to its receptor. Further, insulin deficiency may lead to overproduction of LDL in response to an increased influx of VLDL or its precursor or to impaired removal of VLDL remnants by the liver. Abnormalities in the VLDL particle also may influence conversion to LDL.

Glycated LDL as defects in cholesteryl ester transfer may be found in type

1, as well as in type 2, diabetes.

HIGH DENSITY LIPOPROTEIN

In has been suggested that concentrations of HDL may be low in patients with untreated insulin - deficient diabetes. Response of HDL to insulin therapy is slower than that of VLDL, but HDL increase with the degree of glycaemic control.

One factor responsible for the decrease in HDL in patients with poorly controlled type 1 diabetes is low LPL activity. The reduced activity impairs lipolysis of VLDL and subsequently slows formation of HDL particles[219]. Levels of both HDL cholesterol and phospholipids in type 1 diabetes have been shown to correlate positively with LPL activity; thus, greatly increased catabolism of triglyceride - rich lipoproteins in the presence of excess insulin might augment the HDL compartment. An inverse correlation has been observed between HDL and hepatic lipase activity in the plasma of type 1 diabetic subjects.

TYPE 2 DIABETES MELLITUS

Triglycerides and Very Low Density Lipoprotein

The most common alteration of lipoprotein in type 2 diabetes is hypertriglyceridemia caused by an elevation in VLDL concentrations. In clinical descriptions of diabetic hypertriglyceridemia, an emphasis is often placed on individuals with extremely high levels of plasma and VLDL triglycerides. It is clear, however, from population based studies[220,221] that type 2 diabetes generally is associated with only a 50% to 100% elevation in the plasma levels of total and

VLDL triglycerides.

One of the determinants of diabetic hypertriglyceridemia is the overproduction of VLDL triglycerides[222,223,224], which is most likely due to the increased flow of subtrates, particularly glucose and free fatty acids, to the liver. In addition, individuals with type 2 diabetes appear to have a defect in clearance of VLDL triglyceride that parallels the degree of hyperglycaemia[222,223,224,225]. Studies to date suggest that LPL activity is decreased in individuals with type 2 diabetes, especially those with moderate to severe hyperglycemia who exhibit both insulin deficiency and insulin resistance[226].

Patients with type 2 diabetes have a decreased fractional catabolic rate for VLDL apo B similar to that for VLDL triglyceride[224]. Overproduction of VLDL apoB also occurs in type 2 diabetes and it has been suggested that this overproduction is further increased by obesity[224]. Thus, the extent of overproduction of VLDL triglyceride may be greater than that of apoB in type 2 diabetes, a situation that results in the production of larger triglyceride - rich VLDL particles.

Hyperinsulinemia and the central obesity that typically accompanies insulin resistance also are thought to lead to overproduction and impaired catabolism of VLDL.

Triglyceride elevation in type 2 diabetes may also be due to delayed clearance of postprandial particles[227]. Individuals with diabetes, especially those with severe hyperglycaemia, may have larger triglyceride rich VLDL[224]. This

increased ratio of triglyceride to apoB may be a reflection of a disproportionate influence of type 2 diabetes on VLDL triglyceride production. Subfractions of VLDL have been found to be enriched in the proportion of cholesterol rich particles[228]. These compositional changes may have implications for the increased propensity for atherosclerosis among individuals with type 2 diabetes, because cholesterol - enriched VLDL may be atherogenic. Changes in the distribution of apoE would have important implications for VLDL metabolism in type 2 diabetes because apoE influences the affinity of binding to receptors.

apoE sialation has been reported to be higher in diabetic than non-diabetic individuals, a change that may impair binding to the B/E receptor[229]. Remnant particles from delayed chylomicron clearance may also be present in the VLDL fraction; they are subject to the same compositional alterations discussed for VLDL.

LOW DENSITY LIPOPROTEIN

The density ranges chosen for quantification, of LDL (1.006 to 1.063) result in the inclusion of the IDL fraction. It is possible that the increase in the LDL in type 2 diabetes is the result of an increase in this IDL fraction.

In individual with type 2 diabetes and relatively severe hyperglycemia, the clearance rate for LDL apoB is reduced[224]. Mildly hyperglycaemic individuals with type 2 diabetes may have increased LDL production as well[230]. Because LDL binding is stimulated by insulin[231], defect in LDL clearance in type 2 diabetes may be due to insulin resistance or relative insulin deficiency. This

possibility is supported by the observation that clearance of LDL in type 2 diabetes is positively related to plasma insulin levels and to the insulin response to oral glucose challenge.

Decreased clearance in type 2 diabetes may lead to increased LDL; on the other hand, increased direct removal tends to lower production. These alterations in the flux of both VLDL remnants and LDL particles, coupled with the changes in LDL composition, indicate the LDL in individuals with type 2 diabetes has significant atherogenic potential.

An increase in the proportion of small, dense, triglyceride - enriched LDL has consistently been observed[228]. LDL particles from individuals with diabetes have a decreased ability to bind to receptors, and this decrease in binding is inversely related to the size and ratio of triglyceride to protein in LDL[232]. LDL in diabetic individuals has been shown to be more rapidly oxidized. This may be in part because of the increased oxidative susceptibility of small, dense LDL particles, which are prevalent in diabetic individual also. Oxidized LDL particles are believed to play a minor role in stimulating the atherosclerotic process because of their recognition by macrophage receptors.

Increased plasma triglyceride levels, low HDL levels and small dense LDLs usually occur together in a lipoprotein pattern often referred to as atherogenic dyslipidemia[233]. This abnormal pattern occurs in insulin resistance, is exacerbated in diabetes[234], and is derived in part from alterations in apoB metabolism because triglyceride rich VLDLs are the precursors of denser LDL particles[235]. Small dense LDLs are slowly catabolized because they do not bind

well to the B/E receptor.

Nonenzymatic glycation (or glycosylation) of apoB also may influence LDL metabolism in diabetes. Small, dense LDLs are more rapidly glycated. The extent of glycation of LDL in individuals with type 2 diabetes who have moderate hyperglycaemia is approximately 2% to 5%[236], and this degree of glycation of lysine residues has been shown to decrease LDL catabolism in vivo by 5% to 25%[237]. Glycated LDLs also appear to exhibit altered interactions with endothelial cells, stimulate cytokine production, and enhance cholesteryl ester synthesis in human macrophages[252]. Moreover, glycated LDLs are more readily oxidized. Thus, the glycation of LDL may represent an important mechanism by which atherogenesis is increased in type 2 diabetes. Together, glycation and oxidation render LDLs more immunogenic; the formation of antibody-antigen complexes stimulates macrophage accumulation and further foam cell formation[238].

Finally, a pattern of abnormal cholesterol transport and transfer in the plasma has been shown in patients with type 2 diabetes. The transfer of LCAT-synthesized cholesteryl esters to VLDL and LDL is inhibited, with a concomitant increase in their transfer to HDL; this abnormal metabolic pattern is reversed by insulin therapy. The block in cholesterol ester transfer activity in patients with type 2 diabetes is correlated with an increase in free cholesterol content of both LDL and VLDL. Therefore, in type 2 diabetes, this abnormal cholesteryl ester transfer may be related to an increased risk for atherosclerosis.

HIGH - DENSITY LIPOPROTEIN

Almost as common as the observation of increased VLDL concentrations in type 2 diabetes is the finding of decreased concentrations of HDL cholesterol in individuals with type 2 diabetes.

Individuals with type 2 diabetes have an increased rate of HDL clearance, as measured by apoA_1 and apoA_{11} kinetics[239,240]. Significant correlation have been found between HDL clearance and plasma concentrations of HDL cholesterol and apo A_1, and the increase in HDL clearance was directly related to plasma glucose levels. The finding of increased HDL clearance is consistent with lower VLDL clearance and lower LPL activity. Because HDL concentrations, especially of larger HDLs, increase during the lipolytic process, the decreases in LPL activity and impaired VLDL catabolism have been shown to be correlated with decreases in HDL concentrations in patients with type 2 diabetes. Elevated hepatic lipase activity also may contribute to the decrease in HDL concentrations in type 2 diabetes, because this enzyme also plays a key role in the metabolism of HDL. The changes in lipoprotein and hepatic lipases may act in concert to decrease HDL levels in type 2 diabetes.

Decreased HDL concentrations in type 2 diabetes are reflected mostly in decreases in larger particles. As with LDL, in type 2 diabetes an increased proportion of triglyceride in HDL has been observed. These compositional changes appear to be related to the activity of adipose tissue LPL, because LPL deficiency may be a factor responsible for the altered distribution of HDL particles in untreated type 2 diabetes[241]. Nonenzymatic glycation of HDL appears to interfere with HDL receptor binding . Thus, glycation of HDL may also play a

role in the lower levels of HDL observed in diabetes. Finally, abnormalities in HDL composition have been noted even in individuals with optimal glycaemic control[242]. All of these alterations in HDL composition may impair the role of HDL in reverse cholesterol transport.

Changes in HDL metabolism in insulin resistance include

a. impaired VLDL lipolysis, which depletes HDL from triglyceride-rich lipoproteins to the HDL compartment.
b. Increased activity of hepatic lipase, which facilitates HDL clearance; and
c. Alterations in hepatic function, which inhibit production of apoA_1 (the main apoprotein of HDL) and / or hepatic secretion of nascent HDL[243].

Significant negative relationships between plasma concentrations of insulin and HDL have been observed in subjects with type 2 diabetes as well as a negative relationship between insulin resistance and HDL cholesterol that is independent of insulin or insulin resistance influences the concentration or composition of HDL in some way.

The influence of type 2 diabetes on Lipoproteins are given in Chart No.11.

RELATIONSHIP OF Lp(a) WITH LIPOPROTEIN IN DIABETES MELLITUS

Though much has been reviewed on the effect of glycaemic control on

Lp(a) very little information is available on its association with lipoproteins.

Cristina Hernandez MD et al.,[244] and few others have observed a positive correlation between Lp(a) concentration and LDL cholesterol and an inverse independent association between Lp(a) and TGL. Though the same was also reported by Rainwater[245] and some others in the non diabetic population[11,12,13,14] the influence of LDL cholesterol and triglyceride on Lp(a) was greater in the study of the former on diabetic population than in the non diabetic population of the latter. This was attributed to the higher frequency of lipid disturbances in the diabetic patients as compared to the nondiabetic ones. Cristina Henderson MD et al.,[244] who attributed the levels of Lp(a) more to its rate of synthesis that to its catabolism attributed the following for correlation between the two.

1. Rate of apoB secretion from liver which could be a link between Lp(a) and LDL cholesterol. However Morisett et al.,[246] demonstrated apoB in LDL is synthesized at a rate approximately four times greater than that of the apoB used to produce Lp(a) suggesting that 2 different pools of apo(a) are available for lipoprotein metabolism.

2. The relationship might be observed because LDL cholesterol calculated by the Friedwald formula also contained cholesterol in the Lp(a) particle. This fact is emphasized all the more because it has been reported that in the general population the correlation of Lp(a) and LDL cholesterol disappeared when the contribution of cholesterol of Lp(a) to LDL cholesterol was considered.

AIM OF THE STUDY

On having reviewed about Lp(a) and Diabetes Mellitus, the work of determining Lp(a) and other associated biochemical parameters namely plasma glucose, Total cholesterol, Triglycerides, HDLc, VLDLc and LDLc in the blood of healthy and Type 2 diabetics was taken up with the view of establishing the following.

1. The blood level of Lp(a) in health.

2. To determine whether Lp(a) levels differs from that in health and in Type 2 Diabetic patients who would be classified into 3 groups ie newly diagnosed without treatment and those treated with either oral hypoglycemic agents or insulin.

3. To find out the relationship between Lp(a) with other biochemical parameters in health and the different groups of Type 2 Diabetics patients.

MATERIALS AND METHODS

SUBJECTS

The study was carried out on 87 unrelated individuals who have been living in Tamilnadu for 3 generation. Out of the 87 subjects 20 were from apparently healthy volunteers selected from the staff of MMC or their relatives. The remaining 67 were diabetic patients who were selected from the diabetology OP of Government General Hospital.

Out of 67 diabetic patients selected based on the selection criteria' 3 groups of Type 2 diabetics could be arrived at i.e. 23 of them formed the newly diagnosed group while those on treatment were 44 out of which 22 were Type 2 Diabetics on oral hypoglycemic agents and the other 22 were Type 2 diabetics on treatment with insulin.

Those individuals who were suffering from diseases like HT, Renal failure, liver failure, thyroid dysfunctions, nephrotic syndrome and cardiac pulmonary bypass along with alcoholics and smokers were excluded from the study. None of the subjects were on any drugs with lipid lowering effect.

BLOOD COLLECTION

7ml of blood was drawn from all the above subjects from the anterior cubital vein using sterile disposable syringe. While 1 ml blood was collected into a tube containing the anticoagulant - Oxalate and Fluoride (3:1) for blood sugar estimation, the remaining was poured into another plain tube for serum separation.

The separated serum was centrifuged and from it, 1 ml of clear cell free serum was taken and preserved under - 20°C upto 4 weeks for Lp(a) estimation. From the remaining serum, analysis of serum lipid profile were performed on the same day.

The methodology adopted for the analysis of the various parameters is given below.

1. ESTIMATION OF PLASMA GLUCOSE

Methodology

GOD / POD Method

Principle

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{4 Hydroxy benzoic acid} + \text{4 Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinonemine dye} + 2\,H_2O$$

The intensity of the pink colour formed is proportional to the glucose concentration and can be measured photometrically between 490 to 540 nm.

CALCULATION

$$\text{Glucose} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mg/dl)}$$

Reference Range :

Fasting = 70-110 mg/dl

Post prandial = <140 mg/dl

2. SERUM TOTAL CHOLESTEROL

Methodology

Enzymatic cholesterol oxidase method.

Principle

Cholesterol ester + H_2O ---(Cholesterol esterase)---> Cholesterol + fatty acids

Cholesterol ester + O_2 ---(Cholesterol Oxidase)---> Cholestenone + H_2O_2

$2H_2O_2$+Phenol+4-Aminoantipyrine ---(Peroxidase)---> Red quinone + 4-H_2O

The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone) which is measured at 500nm.

CALCULATION

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Linearity upto 500 mg/dl

RR → 140 to 200 mg/dl

Range depends on age, sex, diet, race and geographical location.

3. SERUM TRIGLYCERIDES

Methodology

Enzymatic calorimetric method.

Principle

Triglycerides + H_2O ---(Lipoprotein Lipase)---> Glycerol+Fatty Acid

Glycerol + ATP ---(Glycerol Kinase)---> Glycerol 3 Phosphate + ADP

Glycerol-3-Phosphate + 0_2 ---(Glycerol-3-Phosphate Oxidase)---> Dihydroxy acetone Phosphate + H_2O_2

2 H_2O_2 + 4 Aminoantipyrine + ADPS ---(Peroxidase)---> Redquinone + $4H_2O$

GPO-Glycerol-3-Phosphate Oxidase
ADPS = N-Ethyl-N-Sulphopropyl-n-anisidine

The intensity of purple coloured complex formed during the reaction is directly proportional to the Triglycerides concentration in the sample and is measured at 546nm.

CALCULATION

$$\text{Concentration of Triglycerides (mg/dl)} = \frac{\text{O.D. Sample}}{\text{O.D.Standard}} \times 200$$

Linearity upto 1000mg / dl

Ref. Range

Male - 60 - 145 mg/dl
Female - 40 - 140 mg/dl

4. HDL - CHOLESTEROL

METHODOLOGY

Phosphotungstic acid method

PRINCIPLES

Chylomicrons, LDL and VLDL were precipitated from serum by phosphotungstate in the presence of divalent cations such as Mg^{2+}. After centrifugation, the cholesterol in the HDL fraction which remained in the supernatant is assayed with enzymatic cholesterol method.

CALCULATION

HDL Cholesterol = (mg/dl)	O.D. Sample --------- O.D. Standard	x Concentration of Std.xDilution Factor

Ref. Range

In Males	30 - 65 mg / dl
In Females	35 - 80 mg / dl

5. VLDL CHOLESTEROL & LDL CHOLESTEROL

VLDL Cholesterol & LDL Cholesterol were calculated using the Friedewald Equation.

Friedewald Equation

VLDLc = TGL / 5
LDLc = Total cholesterol - (HDLc + VLDLc)

Corrected LDL Cholesterol was obtained from the following formula; cholesterol portion of Lp(a), estimated as 0.3 x Lp(a) concentration.

Corrected LDL = LDL Cholesterol -	Cholesterol portion of Lp(a)
Cholesterol portion of Lp(a) =	Lp(a) x 0.3

LIPOPROTEIN (a)

METHODOLOGY

Immunoturbidimetric method

PRINCIPLE

Latex particles coated with antihuman Lp(a) are agglutinated when mixed with samples containing Lp(a). The agglutination causes an absorbance change dependent upon the Lp(a) contents of the patient sample, that can be interpolated in a calibration curve prepared with different calibrators of different Lp(a) contents.

REAGENT COMPOSITION

Lipoprotein (a) R1

Buffer solution (pH 8.3) 1x20ml

Lipoprotein (a) R2

Lipoprotein (a) latex 1x4ml

Lipoprotein (a) Calibrator 1x1ml

LINEARITY

The reagent is linear upto 80 mg /dl. Dilute the sample and repeat the assay. Multiply the result with dilution factor.

PREPARATION AND STABILITY OF WORKING REAGENT

Reagent 1 and Reagent 2 are ready to use. It should be mixed gently before use.

CALIBRATOR

Reconstitute the calibrator with 1 ml of distilled water and this is stable for 7 days at 2-8□C.

Calibration Curve : Prepare dilution of the Lp(a) calibrator using 9g/L saline as diluent.

Dilution	**1**	**2**	**3**	**4**	**5**
Calibrator (µ1)	0	25	50	75	100
Nacl 9g/l (µ1)	100	75	50	25	0
Dilution Factor	0	0.25	0.5	0.75	1.0

Multiply the Lp(a) calibrator concentration by the corresponding dilution factor indicated in the table to obtain the Lp(a) concentration of the different diluted calibrator.

SAMPLE

Fresh serum sample (Free of Hemolysis).

GENERAL SYSTEM PARAMETER

Mode of reaction	-	Fixed time with multi calibrator
Slope of reaction	-	Increasing
Wavelength	-	570 nm (500 - 600 nm)
Temperature	-	37□C
No. of Calibrators	-	5
Calibrator Concentration	-	as on the vial label X Dilution Factor
Linearity	-	80mg/dl
Blank	-	Deionised water
Delay time	-	5 Sec
Interval	-	240 Sec
Sample Volume	-	15µl
Reagent Volume	-	800 µl + 200 µl
Cuvette	-	1 cm light path

LABORATORY PROCEDURE

	Blank	**Calibrator**	**Sample**
Calibrator	-	15 µl	-
Sample	-	-	15 µl
R1 Buffer	800 µl	800 µl	800 µl
R2 Latex	200 µl	200 µl	200 µl

Mix and read the absorbance against blank after 10 seconds (A1) and after 4 minute (A2) of the latex addition.

CALCULATION

Calculate the absorbance difference (A2 - A1) of each diluted Lp(a) calibrator and plot the values found against the Lp(a) concentration in a calibration curve. Lp(a) concentration in the sample is calculated by interpolation of the absorbance difference value (A_2 - A_1) of the sample on the calibration curve.

NORMAL RANGE

It is recommended that each laboratory establish its own reference values. The following values may be used as guideline.

Serum upto 30 mg/dl.

RESULTS

The levels of Lp(a) and other biochemical parameters namely plasma glucose, Total cholesterol, TGL, HDLc, LDLc and corrected LDLc in the blood of all the subjects irrespective of the group to which they belong have been indicated in Master Table No.1. The Table No.2 comprises the results of all the biochemical parameters in the 20 subjects who were apparently normal healthy volunteers. The first 9 subjects of the above table are females while the latter 11 are males. In Table No.3 the results of all the diabetic patients have been pooled together irrespective of the diabetic group to which they belong. Table No.4, 5, 6 comprise the results from 23 patients of Type 2 Diabetes who were newly diagnosed and not on any treatment, 22 patients from Type 2 diabetes on Oral Hypoglycemic agents and 22 patients from Type 2 Diabetes on Insulin therapy respectively. In the above tables from 2 to 6 the calculated mean level and the standard deviation of each parameter is also given. The above mean level of each parameters in the different group is also represented as bar diagrams from Fig.No.7 to 9.

To find out whether differentiation in sex influences the level of Lp(a) or any other biochemical parameter analyzed, the apparently normal healthy males and females of Table No.2 were tabled separately in Table No.7 and 8 respectively.

The mean and standard deviation of the parameters in males when compared with that of females in Table No.9 revealed statistically that there was no appreciable change in the level of any of the parameters with respect to sex. Hence irrespective of sex the mean obtained in Table No.2 has been selected for

all the biochemical parameters as the Reference range for the study.

To find out how far Lp(a) and other parameters varied from the reference range in Type 2 Diabetic groups the Mean and Standard deviation of each parameter in all the 3 groups of Type 2 Diabetics were compared with that of Reference Range in Table No.10, 11, 12 respectively.

To determine the difference in the parameters among the Diabetic groups analysed intercomparison of the results obtained in the different groups of Diabetes was undertaken in Table No.13, 14 and 15. To get at the overall perspective of the parameters statistical variation between controls and diabetics, the mean and standard deviation of the parameters in the entire group of diabetics obtained from Table No.3 have been compared with that of the Reference Range in Table No.16.

Statistical significance has been derived for each parameter in the comparison tables from the p-value obtained which has been calculated using the student t-test.

To obtain the correlation of Lp(a) with lipid parameters pearsons correlation co-efficient were arrived at for each parameter in control and Diabetics separately and together for the entire 87 subjects analyzed irrespective of the factor whether diabetics or not. The results of pearson's correlation have been tabled for controls and diabetics separately in Table No.17 to 23 and for all the subjects together in Table No.24.

DISCUSSION

To start with, the validity of the reference range (RR) obtained for the tested biochemical parameters of serum from Table No.2 which are 77.85 ± 13.05 mg/dl for plasma glucose, 18.69 ± 8.87 mg/dl for Lp(a), 181.6 ± 24.15 mg/dl for Total Cholesterol, 131.10 ± 55.65 mg/dl for TGL, 43.35 ± 5.04 mg/dl for HDLc, 26.4 ± 11.21 mg/dl for VLDLc, 111.85 ± 25.5 mg/dl for LDLc are scrutinized.

The mean level of 18.69 ± 8.87 mg/dl of Lp(a) obtained from controls is well within 15 - 20 mg/dl quoted by Berg et al., and that of Harlampos J Milionis et al.,[123] who have given a Reference Range of less than 30 mg/dl. Similarly even in the kit methodology adopted for its analysis a level of less than 30 mg/dl has been specified as the Reference Range. This level of 18.69 ± 8.87 mg/dl of Lp(a) is however higher to the Reference Range of 4 mg/dl of James A Hearn[92] and 13 to 16 mg/dl obtained by Devanapalli et al.,[193] who had worked on Asian Indians.

The different levels of reference range of Lp(a) obtained by the various workers in the field can be attributed to the fact that plasma Lp(a) level is said to be heritable and there is striking difference in its normal levels in various population inspite of the fact that Lp(a) in blood is constant at any stage of life[72] whether it be newborn, adult or oldage. Moreover it has been reviewed that diet can influence Lp(a) levels[247]; hence the varied dietary habits of the different races of people who had been worked on by the above scientists and the author can also be a contributory factor for the different Reference Range of Lp(a) obtained by each.

The Reference Range obtained for the other routine biochemical parameters correlate well to that quoted in standard text books and of course with

the Reference Range of kit methodologies selected for evaluating their concentrations. Therefore the above mean of each parameter including Lp(a) are found to be valid and hence acceptable as Reference Range for the study.

Comparison of the mean levels of biochemical parameters of Newly diagnosed Type 2 Diabetics who have not been initiated any treatment for it with that of controls (Table No.10) shows a S ↑ in Lp(a) (p=0.05) and a HS ↑ of plasma glucose (p = 0.001), TGL (p=0.01) and VLDLc (p=0.01). Similar comparison of Type 2 Diabetics on treatment with Oral hypoglycemic agents with that of controls (Table No.11) reveals in addition to the statistical significance of the former table S ↑ of Total Cholesterol (p=0.023), and LDLc (p=0.05) and HS ↓ of HDLc (p=0.001). Comparison of the mean levels in Type 2 Diabetics on insulin treatment with that of controls (Table No.12) shows that there is HS ↑ of Lp(a) (p=0.001) similar to that of plasma glucose (p=0.001), TGL (p=0.009) and VLDLc (p=0.01) together with a S ↑ of serum Total cholesterol (p=0.024) and LDLc (p=0.05) against a S ↓ of HDLc (p=0.031).

HS increase of plasma glucose in the above 3 Tables is natural as the comparison is between diabetics and the non - diabetic healthy.

The increase of Lp(a) level in diabetics from its level in healthy controls which is obvious in Table No.10, 11, 12 is not a surprise to the author for there are several literature evidences that in Diabetes mellitus whether it be in Type 1 or 2 there is increase of Lp(a).

The observed increase of Lp(a) in all 3 groups of diabetics analyzed can be

attributed to be due to the following reasons :

1. As the diabetic groups analyzed belonged to Type 2 Diabetes Mellitus where peripheral resistance to the action of insulin is the main causative factor, hyperinsulinemia will prevail in the above groups. As it has been reviewed that chronic hyperinsulinemia can increase Lp(a) level[120], the increase of Lp(a) in Diabetics of our study can be said to be contributed by the prevailing hyperinsulinemia.

2. Increase in the rate of synthesis of Lp(a) as suggested by Cristina Hernandez et al.,[244] who have stated that the Lp(a) level is dependent more on the rate of its synthesis than on its catabolic rate.

3. As a link between Lp(a) and LDL through apoB 100 has been established by Morrisett et al.,[246] it is inferred that there should be increased rate of secretion of apoB 100 from the liver which will contribute towards the increase of LDL and Lp(a).

4. Decreased rate of catabolism of LDL in diabetics[224,231] for which the reasons have been already enumerated. As Lp(a) is constituted by apo(a) and LDL, decrease in the catabolism of the latter will be naturally reflected on the level of Lp(a).

5. Moreover it has been reviewed that Lp(a) is catabolized by the same receptor by which LDL is catabolized. Hence Lp(a) sharing the same receptor as LDL for its catabolism will be naturally increased because it has been found by Cristina Hernandez et al.,[244] that LDL has a higher

affinity for the receptor than Lp(a). Hence when LDL level is increased in diabetics it all the more will compete with Lp(a) for the receptor. It has also been said that two different conformation of receptor are present for binding to Lp(a) and LDL. Therefore it can also be assumed that the conformation of the receptor for binding to LDL becomes more prominent in conditions where LDL levels increase. This will decrease the catabolism of Lp(a) through this route and eventually increase its level in blood. Though the exact percentage of Lp(a) catabolized through the receptor pathway is not known, the receptor affinity for Lp(a) can be altered in Diabetics and thereby lead to an increase of Lp(a).

Apt to the statement reviewed in literature, the most common early alternation of lipoproteins in Type 2 Diabetes is hypertriglyceridemia resulting from evaluation of VLDL concentration, there is a HS ↑ of TGL and VLDLc in all the 3 groups of Type 2 Diabetes Mellitus from their Reference Range. This increase of TGL and VLDLc in Type 2 Diabetes can be due to the following facts;

1. Overproduction of substrates particularly glucose and free fatty acids to liver.

2. Defects in clearance of VLDL triglyceride[224] which is said to parallel the degree of hyperglycemia.

3. Decrease in activity of LPL[226] which normally hydrolyzes triglycerides in lipoporteins in VLDL and chylomicrons.

4. Overproduction of VLDL apoB and decrease in its fractional catabolic rate.

5. Alteration in the composition of VLDL ie VLDL triglyceride production is disproportionately influenced in resulting in large triglyceride rich VLDL which have increased ratio of TGL : apoB. As a result of these molecules there is increase of VLDL and TGL.

The significant increase of LDL cholesterol and Total cholesterol in Type 2 Diabetics can be the result of the following reasons.

1. Defect in LDL clearance which may be due to insulin resistance or relative insulin deficiency[250]. This is said to be due to decrease in LDL binding to its receptor which is normally stimulated by insulin.

2. Reduction in the clearance rate for LDL apoB[250].

3. Increase in the proportion of small dense triglyceride enriched LDL which has decreased ability to bind to receptors[250].

4. Nonenzymatic glycation of apoB of LDL especially small dense LDL which decreases LDL catabolism[250]. It is said that glycated LDL's more than catabolism are prone for oxidation whereby oxidised LDL is formed.

5. Block in cholesterol ester transfer activity from HDL to VLDL and LDL with an increase in free cholesterol in the latter two[250].

On the other hand decrease in HDL cholesterol in Type 2 Diabetes can be

attributed to the following reasons.

1. Increased rate of HDL clearance[250].

2. Elevated hepatic lipase activity which facilitates its clearance and thereby contributes to the decrease of HDL concentration[250].

3. Decrease in LPL activity and impaired VLDL catabolism decrease the concentration of HDL especially larger HDL which normally increase during lipolytic process[250].

4. Alteration in hepatic function which inhibits the production of apoA_1 or hepatic secretion of nascent HDL[250].

5. Occurrence of increased proportion of TGL in HDL as a result of the activity of adipose tissue LPL, alters the catabolism of HDL[250].

Absence of any significant change in Total cholesterol, LDLc or HDLc in Newly Diagnosed patients of Type 2 Diabetes which is contrary to the changes observed in patients treated with OHA or Insulin can be attributed to the long standing duration of the disease in the latter two groups to that of the former group. As reviewed, only the earliest lipoprotein change namely increase of TGL and VLDLc is seen in the newly diagnosed group of Type 2 Diabetic and hence it may be presumed that the subjects belonging to this group have attended the Diabetic OP during the early phase of the disease itself. Therefore eventhough the plasma glucose level of this group of Type 2 Diabetes Mellitus is higher to the other two group of treated Type 2 Diabetes mellitus it has not significantly

affected the other lipid parameters. Corrected LDLc does not show any significance in the above 3 comparative tables. The absence of any SS for corrected LDLc inspite of a S ↑ of Lp(a) in Table No.11, 12 suggests that the LDLc increase is the result of the additional cholesterol of Lp(a). So when this fraction is deducted from LDLc to give the corrected LDL the SS disappears.

In comparison of the 3 groups of Diabetics with each other in Table No.13, 14 and 15 reveals that plasma glucose levels are lower in both the treated groups to that in the newly diagnosed groups, the degree of lowering being statistically significant. Similarly the levels of HDLc is lower in Type 2 diabetes treated with OHA than to its level in the Newly diagnosed group. However statistical significance to the extent of HS has been obtained only between the HDLc of the Diabetic group on OHA and the Newly diangosed group. Though Barbara V Howard and Wm James Howard[248] have stated that improvement in glycaemic control elevates HDLc, the glycaemic control achieved by the diabetic patients of this study is not sufficient to increase the HDLc level to the level in the Newly Diagnosed group which is near normal.

The mean level of the assessed parameters of all the 67 diabetic patients compared with that of controls (Table No.16) shows a statistical increase in the level of all the parameters in diabetics except LDL which is just significant.

On analysis of the correlation of Lp(a) with other parameters we find that Lp(a) does not correlate to the plasma glucose and that the degree of glycaemic control does not influence plasma Lp(a) levels either in controls or any of the Diabetic groups analyzed. This finding correlates well to the study of A Perez et al.,[209] though his work has been carried out in Type 1 diabetic patients. The

absence of any significant correlation between Lp(a) and blood sugar in control and diabetics is evident in Table No.17a and 17b.

Correlation of Lp(a) with other lipid parameters has revealed a positive HS (p=0.001) correlation between Lp(a) and Total cholesterol in Diabetics (Table No.18b). This was however absent in controls (Table No.18a) of the study. On the other hand TGL (p=0.01) showed a negative correlation with Lp(a) in Diabetics (Table No.19b) with absence of any correlation between the two in controls (Table No.19a). Among the fractions of cholesterol in the various lipoproteins HDLc does not correlate to Lp(a) either in controls or Diabetics (Table No.20a & 20b); but VLDLc (p=0.015) and LDLc (p=0.001) have negative S correlation and positive HS correlation respectively with Lp(a) in the Diabetics (Table No.21b and 22b) which is absent in their counter part controls (Table No.21a and 22a). Corrected LDL (p=0.001) also has a similar correlation as that of LDLc with Lp(a) (Table No.23b). However the correlation of levels of Lp(a) in the entire 87 subjects irrespective of the fact Diabetic or not proves that only Total cholesterol (p=0.001), LDLc (p=0.001) and corrected LDL (p=0.001) have a positive HS correlation with Lp(a) (Table No.24). As LDLc is the main lipid fraction of LDL and as LDL is a component of Lp(a) the author is not surprised to see the positive HS correlation of LDLc or corrected LDLc. Moreover as LDLc is the major fraction of the Total cholesterol the HS positive correlation of Lp(a) with LDLc is also reflected on Total cholesterol.

Since the level of the main biochemical parameters of this study namely Lp(a) differs statistically by a HS degree between controls and diabetics attempt is made to arrive at a cut off level for this parameter between the two groups. For this purpose the levels of Lp(a) in the 20 controls and 18 of each group of Type 2

diabetes are plotted as line diagram in Graph No.1.

Various cut off levels have been selected from the graph between controls and the diabetic and the sensitivity, specificity, positive predictive value and negative predictive value have been calculated and shown in Table No.25. From this table it is clear that 25 mg/dl of Lp(a) is the most appropriate cut off level to demarcate between controls and Type 2 Diabetes. The same has also been depicted in Graph No.1.

CONCLUSION

From the results on the study on 87 subjects consisting of 20 control, 23 Type 2 diabetic patients who were newly diagnosed and not on any treatment, 22 Type 2 diabetic patients on oral hypoglycemic agents and 22 type 2 diabetic patients on insulin therapy in whom serum Lp(a) along with plasma glucose, serum total cholesterol, serum triglycerides, serum HDLc were analyzed and VLDLc, LDLc and corrected LDLc were calculated the following facts are revalued.

1. Reference range for serum Lp(a) in the study is 18.69 ± 8.87. Difference in sex does not alter the RR of Lp(a).

2. Serum Lp(a) is elevated in Type 2 diabetics. The increase is found in Diabetic irrespective of whether newly diagnosed not on treatment or old cases on treatment with OHA or insulin.

3. Serum Lp(a) does not correlate to plasma glucose i.e to the level of glycaemic control.

4. Serum Lp(a) does not correlate with any of the lipid parameters in controls.

5. Serum Lp(a) positively correlates with Total cholesterol, LDLc, and corrected LDLc and negatively with TGL and VLDLc in diabetics.

6. The most appropriate cut off level of Lp(a) between controls and diabetics is 25 mg/dl.

SCOPE FOR FURTHER STUDY

1. Lp(a) assessment can be done during various age groups from birth.

2. Lp(a) levels of siblings can be correlated with parents to find out the degree of inheritance.

3. Lp(a) assessment between premenopausal and post menopausal women.

4. Lp(a) can be assessed in Type 2 DM with mild glycaemic control, moderate glycaemic control, tight glycaemic control.

5. To study the association of Lp(a) and the development of Diabetic complications.

6. To determine the concentration of the different types of LDL in diabetes that promote atherosclerosis.

7. To find out Lp(a) levels type 1 diabetes patients.

8. Lp(a) can be assessed in various grades of coronary heart diseases.

9. Lp(a) assessment can be done in relation to other risk factors in AMI.

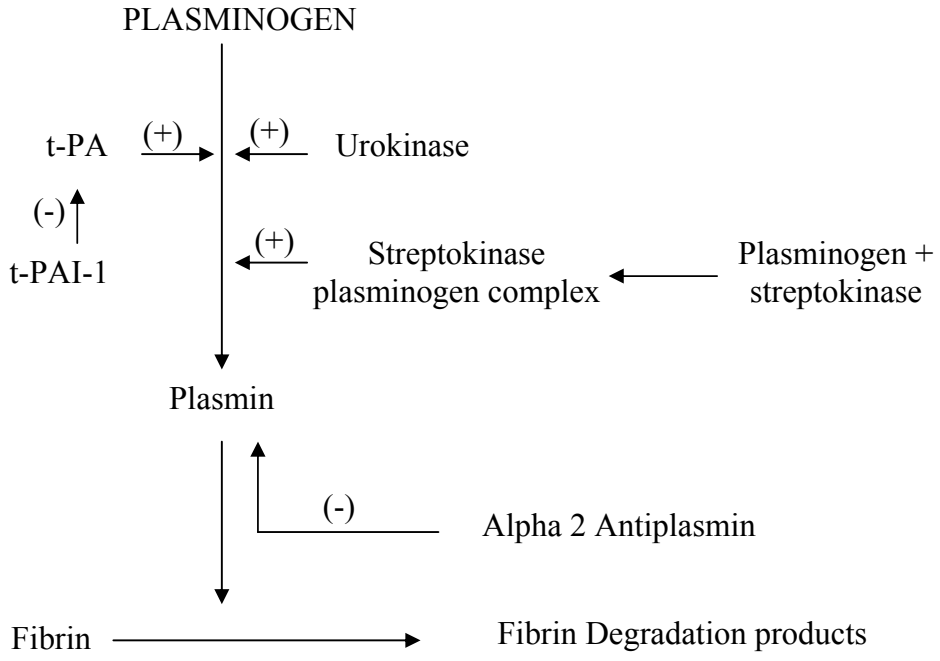
10. Lp(a) can be assessed from the time of onset of AMI through the course of the disease, to findout its relevance to treatment and to determine whether it can be used as a prognostic indicator.

11. Lp(a) assessment can done in relation to other risk factors in peripheral arterial diseases.

12. Lp(a) assessment can be done in relation to stroke and other risk factors in stroke individuals.

13. To assess Lp(a) levels in other diseases where it is said to be increased stroke, Nephrotic syndrome, ESRD etc.

CHART NO.4
CASCADE OF REACTIONS INVOLVED IN FIBRINOLYSIS



Courtesy : Harpers Biochemistry - 24th edition Fig.59 - 16, (p.728)

CHART NO.5
INFLUENCE OF Lp(a) ON FIBRINOLYSIS

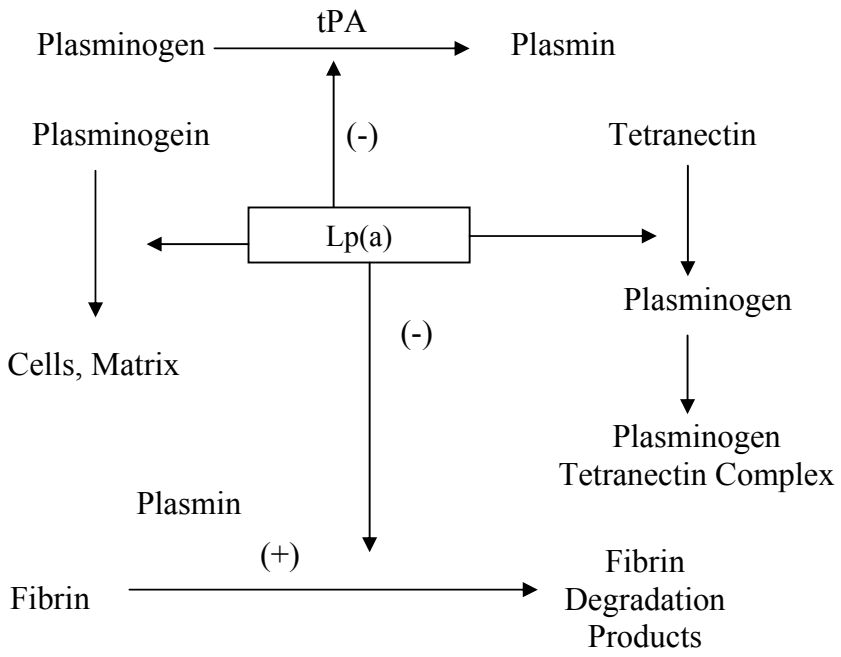


CHART NO.8

A SCHEMATIC REPRESENTATION OF Lp(a) CATABOLISM

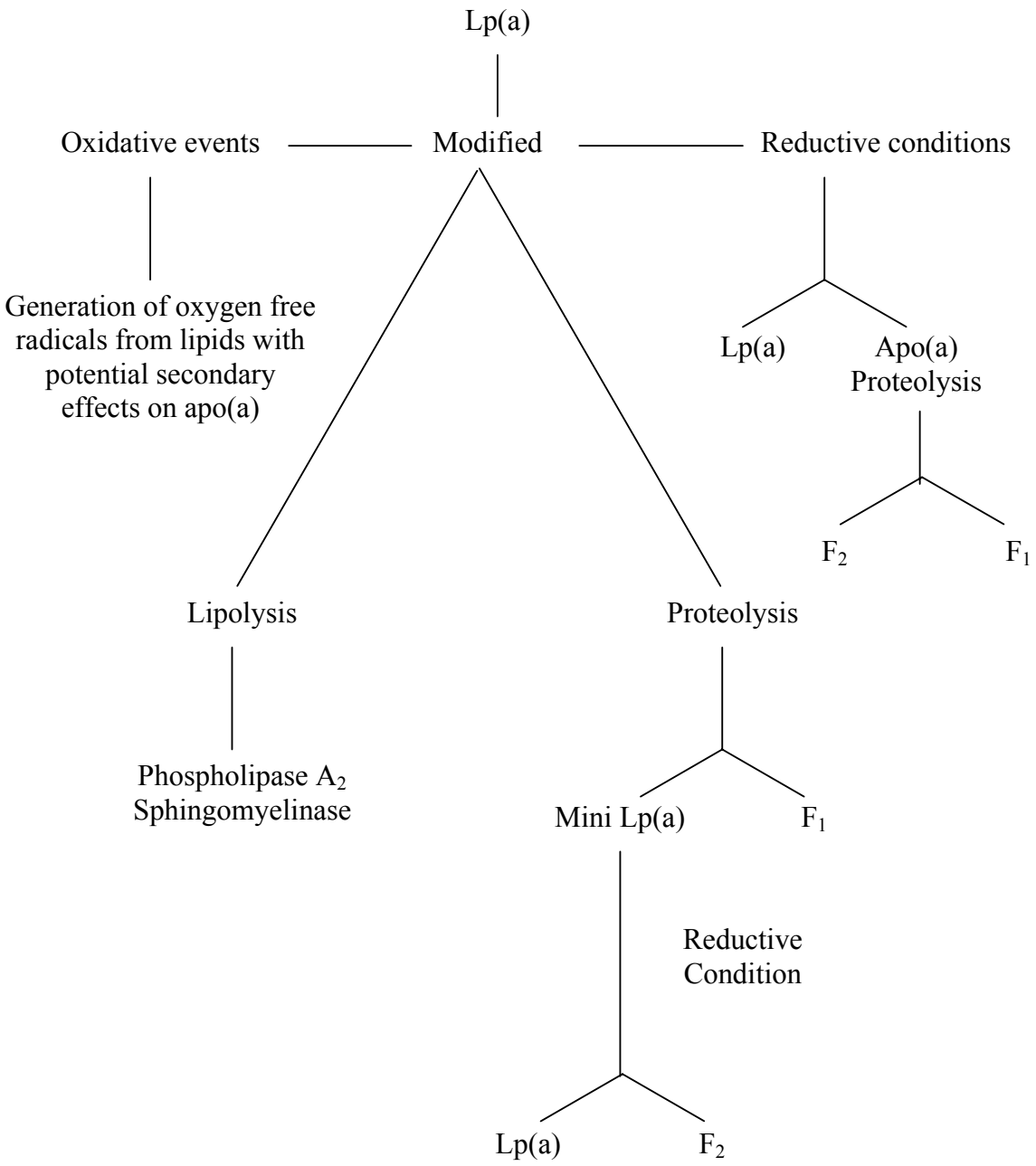


CHART NO.3

ROLE OF Lp(a) IN TISSUE REPAIR AND FIBRINOLYSIS

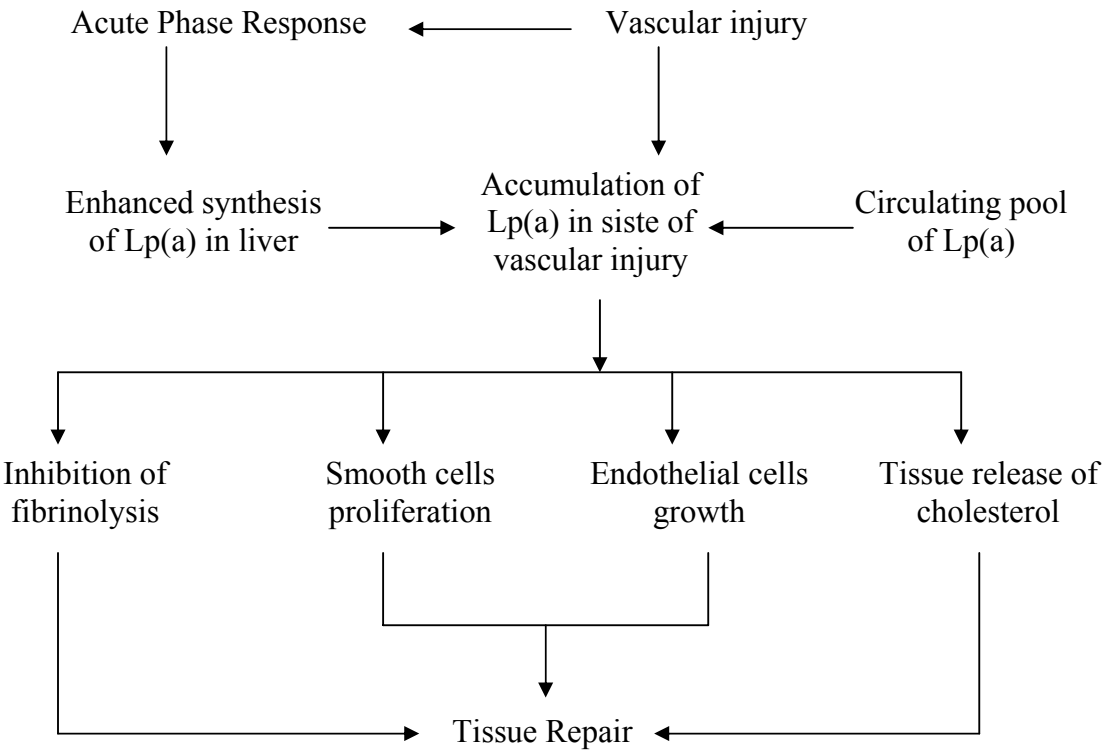
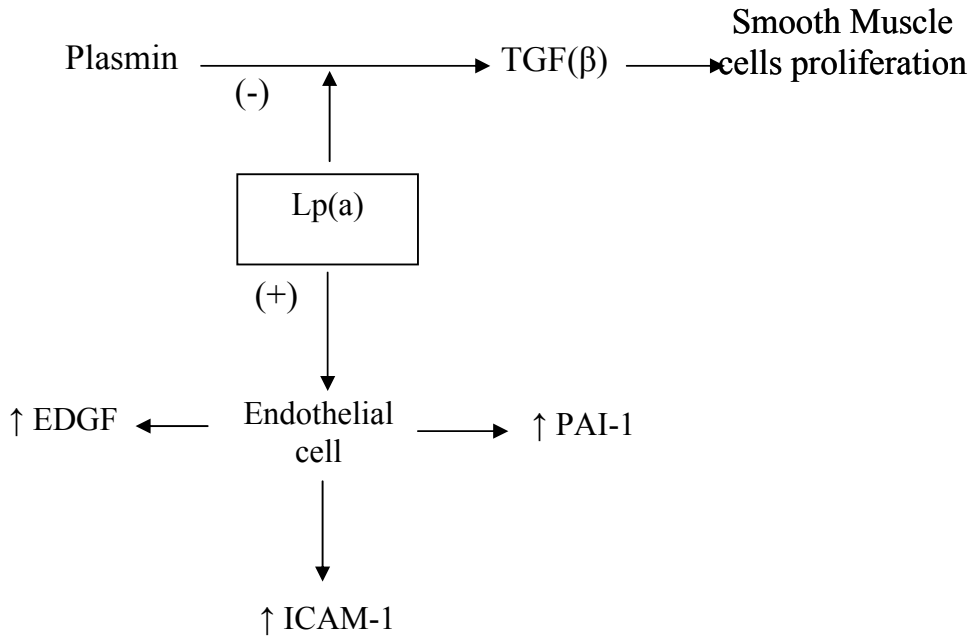


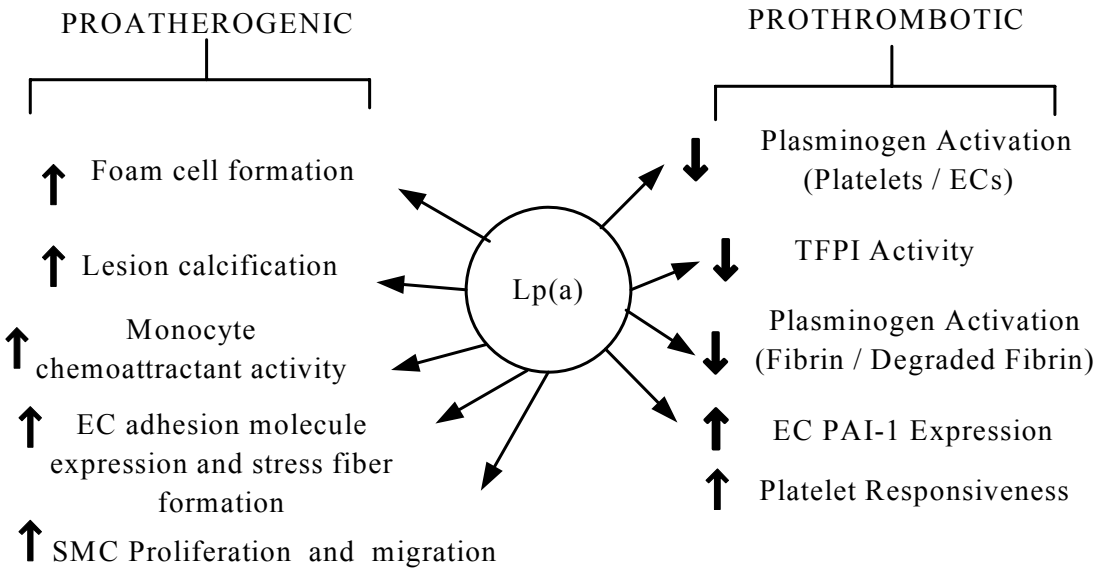
CHART NO.6

Lp(a) AND ATHEROSCLEROSIS



EDGF	:	Endothelial derived growth factor
PAI-1	:	Plasminogen Activator inhibitor - 1
ICAM - 1	:	Intercellular adhesion molecule - 1
TGF - β	:	Transforming Growth factor - β

CHART NO.7



Potential Mechanisms of Lipoprotein(a) (Lp(a)) pathogenecity. A variety of potential mechanisms that may account for the association of elevated Lp(a) levels with atherothrombotic disease has been described. These can be divided into those that are proatherogenic and those that are prothrombotic. (EC; Endothelial Cell, PA1-1; Plasminogen Activator Inhibitor, SMC; Smooth muscle cell, TFP1 ; Tissue Factor Pathway Inhibitor).

CHART NO.2

ISOFORMS OF Apo(a)

Designation	No	Molecular Mass (kDa)	K IV Repeat Nos.	Conc. of Lp(a) in mg/dl
F	1	< 450	11 - 13	-
B	1	~ 500	14 - 16	61.7
S	4	-	-	-
S1	-	~ 550	17 - 19	34.4
S2	-	~ 600	20 - 22	24.5
S3	-	~ 650	23 - 25	10.2
S4	-	> 700	26 - 42	<5.7

FIGURE NO.5

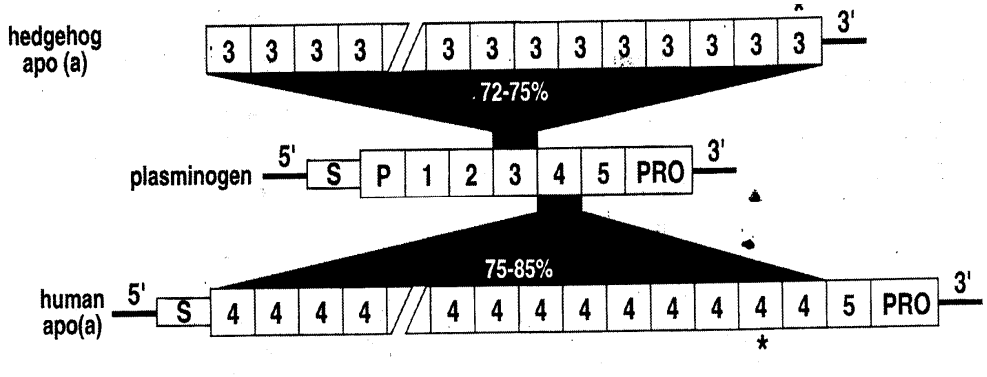
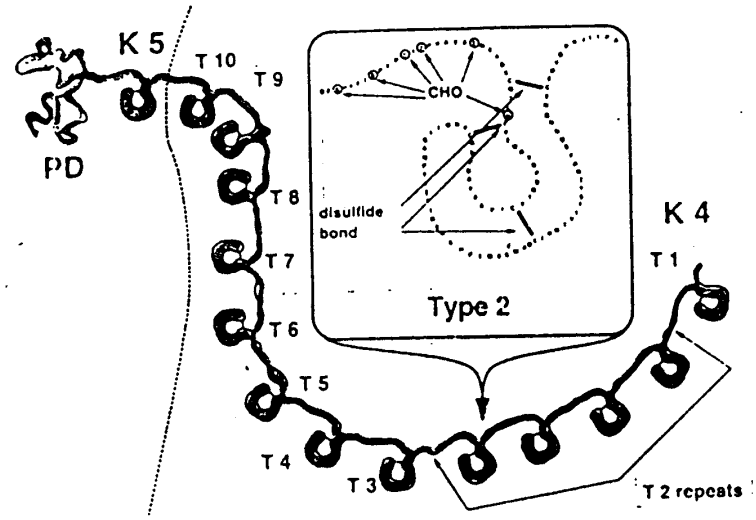


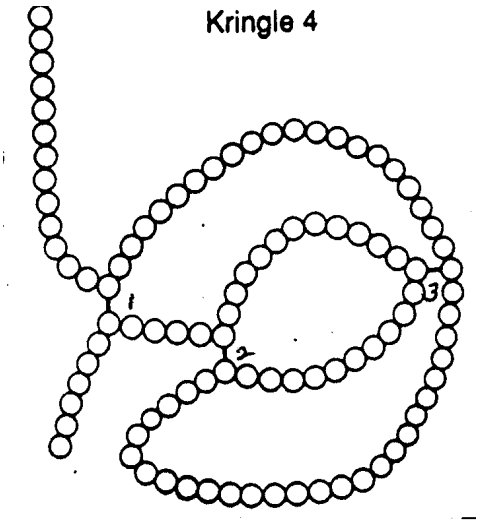
FIGURE NO.3
KRINGLE DOMAINS OF Apo(a)



K-4 Kringle 4
K-5 - Kringle 5
T1-10 - Types 1-10 of K4
PD - Protease Domain

Courtesy : Clinical Chemistry (Tietz)

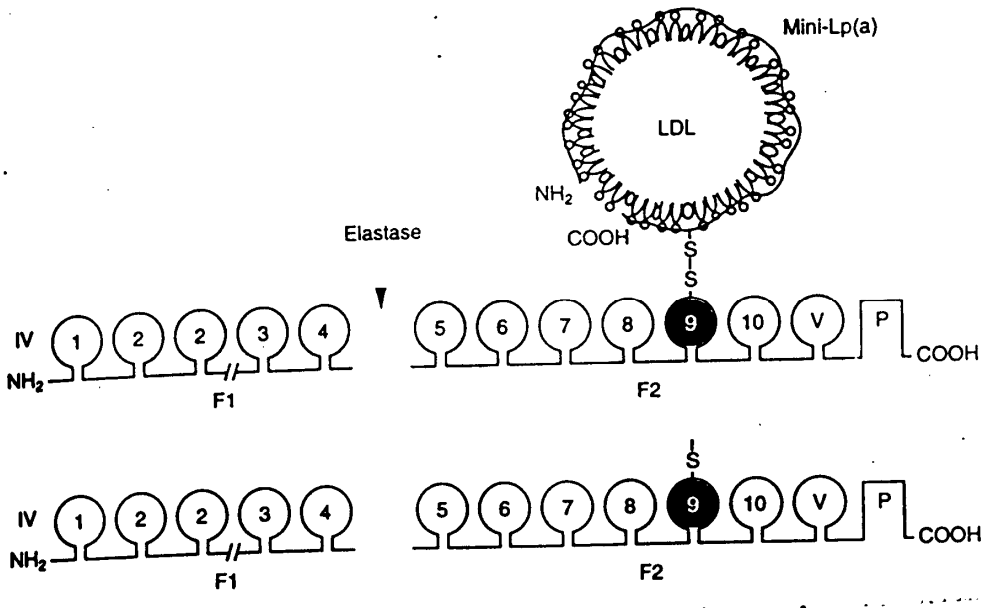
FIGURE NO.4
STRUCTURE OF KRINGLE DOMAIN OF Apo(a)



1, 2, 3 - Crosslinking disulphide bridges
Courtesy : British Heart Jor, 1991

FIGURE NO.6

CATABOLIC PRODUCTS OF Lp(a)

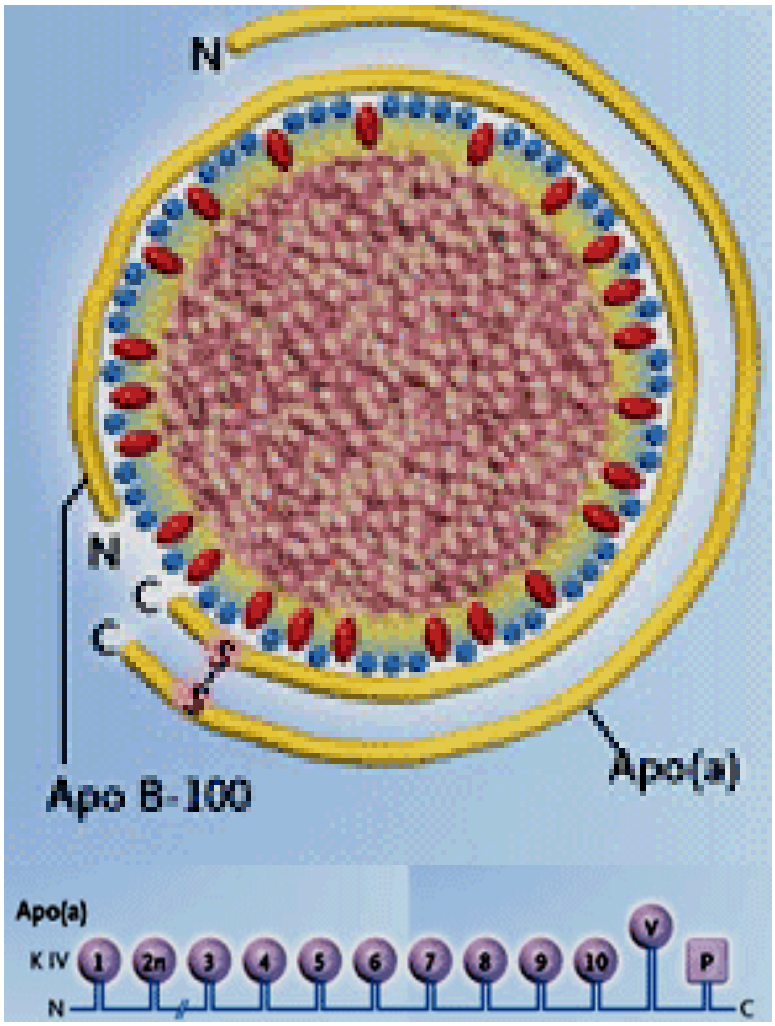


F1	-	Fragment 1
F2	-	Fragment 2
IV	-	Kringle IV
V	-	Kringle V
LDL	-	Low Density Lipoprotein
P	-	Protease domain

Courtesy : American Journal of Cardiology, 1998

FIGURE NO.2

LIPOPROTEIN (a)



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